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THE NATURE AND INTERACTION OF GENES CONDITIONING REACTION TO RUST IN FLAX [1]

By W. M. MYERS [2]

Assistant in agronomy and plant genetics, Minnesota Agricultural Experiment Station

INTRODUCTION

Flax rust, caused by *Melampsora lini* (Pers.) Lév. is widespread throughout the flax-growing regions of the world, and in certain years epiphytotics of serious proportions occur, causing severe losses to growers. The threat of rust epiphytotics in Minnesota has been sufficiently great to be a major factor in causing the removal from the recommended list of the two susceptible varieties Chippewa and Winona, and at the present time makes it imperative that new varieties of flax to be recommended for commercial production be highly resistant to, or immune from, rust.

The physiologic-form concept has served to emphasize the necessity for producing varieties resistant to all races of the pathogen prevalent in a locality. Consequently, the inheritance of reaction to a collection of rust is of primary importance in breeding for rust resistance in flax. Likewise, a knowledge of the degree of association between the reaction of hybrids to a collection of rust and to a single physiologic race commonly found in the region is of considerable importance both from a practical and from a theoretical viewpoint.

The purpose of this study was threefold: (1) To determine the nature of interaction of genes conditioning different types of rust reaction; (2) to determine the relationship and interaction of genes conditioning a similar reaction type in different varieties; and (3) to determine the relationship between the reaction of hybrids to a collection of rust and to a single physiologic race.

LITERATURE REVIEW

Studies of the inheritance of disease resistance in crop plants have been a fruitful field for the application of genetic principles to plant breeding, and the literature pertaining to such studies is voluminous. Likewise, the morphological characters of flax have been the subject of rather extensive genetical researches, particularly by Tammes. This literature has been reviewed in recent papers by Tammes (*9*, *10*)[3] and the writer (*7*). It is a singular fact, however, that relatively little work has been reported on the inheritance of reaction to rust in flax.

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[3] Reference is made by number (italic) to Literature Cited, p. 666.

In 1921, Henry (*5*) stated that several previous investigators had observed a differential rust reaction in different varieties of flax, some reporting immune varieties. At the same time, he reported that numerous varieties had been immune from rust in tests in Minnesota. Later, (*6*) he stated that Ottawa 770B, Argentine Selection, and Bombay had remained consistently immune when thoroughly tested with several collections of rust from the United States and Canada, and that all three had likewise been immune when inoculated with a collection of rust furnished by Tammes from her flax-breeding gardens at the University of Groningen, Groningen, Netherlands. In addition, Henry (*6*) stated that Hiratsuka tested Ottawa 770B and Argentine Selection in Japan with the same results.

Henry (*6*) studied the inheritance of immunity in crosses involving Argentine Selection, Ottawa 770B, and Bombay as the immune parents. The immunity of Ottawa 770B and Bombay was in each case dependent upon a single dominant factor. In Argentine Selection, apparently two dominant factors were present, either of which conditioned immunity. In crosses involving Ottawa 770B, rust reaction and flower color were inherited independently.

Recently, Flor (*3*) has reported the differentiation of 14 physiologic forms of *Melampsora lini* by the use of seven varieties of cultivated flax. Of the 165 varieties of flax inoculated with forms 1 to 5, only 13 gave indications of being rust differentials, the remaining 152 showing no specific response to the 5 forms of rust. Flor states that the lack of genetic purity of the varieties with regard to rust reaction was one of the striking features of his results.

MATERIAL AND METHODS

The following varieties of flax (*Linum usitatissimum* L.), used as parents in the crosses reported in this paper, are grouped according to their supposed rust reaction at the time this study was outlined:

Immune: Ottawa 770B, C. I. 355 [4]; Newland, C. I. 188; Minnesota Selection, C. I. 438; Long × E, C. I. 697; Pale Blue Verbena, C. I. 416–3; and Argentine Selection, C. I. 712.

Resistant: Light Mauve, C. I. 379–1; Pale Pink, C. I. 649; Bison, C. I. 389; Redwing, C. I. 320; and Ottawa 829c, C. I. 391.

Moderately susceptible: Bolley Golden, C. I. 644; Pale Blue, C. I. 423; and Abyssinian Yellow, C. I. 300.

Susceptible: Common Pink, C. I. 479.

The data on rust reaction, used in outlining this study, were made available to the author by C. C. Allison of the Division of Plant Pathology and Botany, University of Minnesota, and were from notes taken on the varieties grown at the Coon Creek Experimental field in 1932.

The parental material, wherever possible, was taken from rows descending from individual plants. However, such rows were not available of Newland, Long × E, C. I. 416–3, C. I. 712, Light Mauve, and Bolley Golden, and for these varieties it was necessary to plant bulk seed for making crosses. A total of 37 crosses was made. Varieties representing different types of rust reaction were intercrossed, and, in addition, varieties showing a similar type of reaction were crossed in all combinations. The crosses were made in the greenhouse during

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

the winter of 1933–34, and the parents and F_1 plants were grown on peat soil at the Coon Creek experimental field in 1934. In addition, an F_2 generation of Ottawa 770B × Pale Blue, and F_2 and F_3 generations of Ottawa 770B × Redwing were grown in space-planted rows. Additional hybrid seed of each of the crosses was obtained in the flax-crossing plot in 1934.

F_2 generations of all crosses were grown in 1935 at University Farm, Minn., and on the Coon Creek peat. However, because of adverse climatic conditions, no data were obtained on rust reaction of the hybrid material during this summer.

In the greenhouse studies of rust reaction, conducted during the winter of 1935–36, F_1 and F_3 lines were used in all crosses where seed was available. In certain crosses where there were insufficient F_3 lines F_2 material was also used. Each F_3 line was planted in a single 4-inch pot at the rate of 20 seeds per line where sufficient seed was available. Likewise, the F_2 generations and the parental checks were planted at the rate of 20 seeds per 4-inch pot.

The collection of rust was obtained at University Farm from the variety Winona, previously inoculated with rust collected at Coon Creek. Physiologic form 4 was obtained from H. H. Flor, of the United States Department of Agriculture. Both the collection and the single form were increased in the greenhouse on Winona.

A temperature of about 70° F. was found to be suitable for growing the flax and for producing good rust infection. Two large incubators, each with a capacity of seventy-five 4-inch pots, were available and an incubation period of 48 hours was used. As a result, about 150 pots were planted every second day. The first planting was inoculated by the brushing method, with the collection of rust, 3 weeks after the date of planting. The plants were then 4 to 6 inches tall. Inoculations were continued every other day on the subsequent plantings until in November, when the low light intensity and duration retarded the growth of the flax. Since it seemed desirable to inoculate all crosses at nearly the same stage of growth, the period from planting to inoculation was gradually extended from 3 weeks to 4 weeks in December. As soon as seed of all the material had been planted for testing with the collection of rust, a duplicate planting was started for testing with the single physiologic race. The procedure followed with the material for inoculation with the single race was essentially the same as that for the collection.

In taking the notes, no standardized classes were available as is the case in seedling studies with the cereals, particularly wheat. Consequently, it was necessary to set up the classes as the notes were taken. Eleven classes were used, based upon extent and type of infection. These classes were as follows:

Class 0: Plants showing no macroscopic evidence of the presence of the pathogen (pl. 1, *A*).

Class 1: Plants with small flecks but no pustules (pl.1, *B*).

Class 2: Plants with flecks and very small pustules, the pustules surrounded by a narrow necrotic area or by chlorotic tissue (pl. 1, *C* and *D*).

Class 3: Plants with flecks, very small pustules, and occasional medium large pustules intermingled on the same leaves over the entire plant. Pustules usually surrounded by a necrotic area although occasional pustules were surrounded by a chlorotic area (pl. 1, *E*).

Class 4: Plants with few small pustules (less than four or five per leaf) surrounded by necrotic areas on older leaves. Leaves on the upper one-third of the plant with large susceptible pustules, surrounded by chlorotic area (pl. 1, F).

Class 5: Plants like class 4 except with more pustules per leaf (pl. 1, *G*).

Class 6: Plants with large pustules surrounded by chlorotic areas on all leaves. Usually two, three, or four pustules per leaf surface (pl. 1, *H*).

Class 7: Plants with reaction type like class 6, except with five to eight pustules per leaf surface (pl. 1, *I*).

Class 8: Reaction type like classes 6 and 7 except with pustules more numerous, causing the chlorotic areas surrounding the pustules to coalesce, thus producing a more or less uniform chlorotic condition where the pustules were thick (pl. 1, *J*).

Class 9: Pustules on older leaves surrounded by narrow "green islands" with intervening chlorosis, giving the leaves a spotted appearance. Large pustules on younger leaves similar to those in classes 6, 7, and 8. Many pustules per plant (pl. 1, *K*).

Class 10: Reaction type like class 9 except with few pustules per leaf (pl. 1, *L*).

FIELD STUDIES

The rust reaction of the parental varieties grown at Coon Creek during the summers of 1934 and 1935 are presented in table 1.

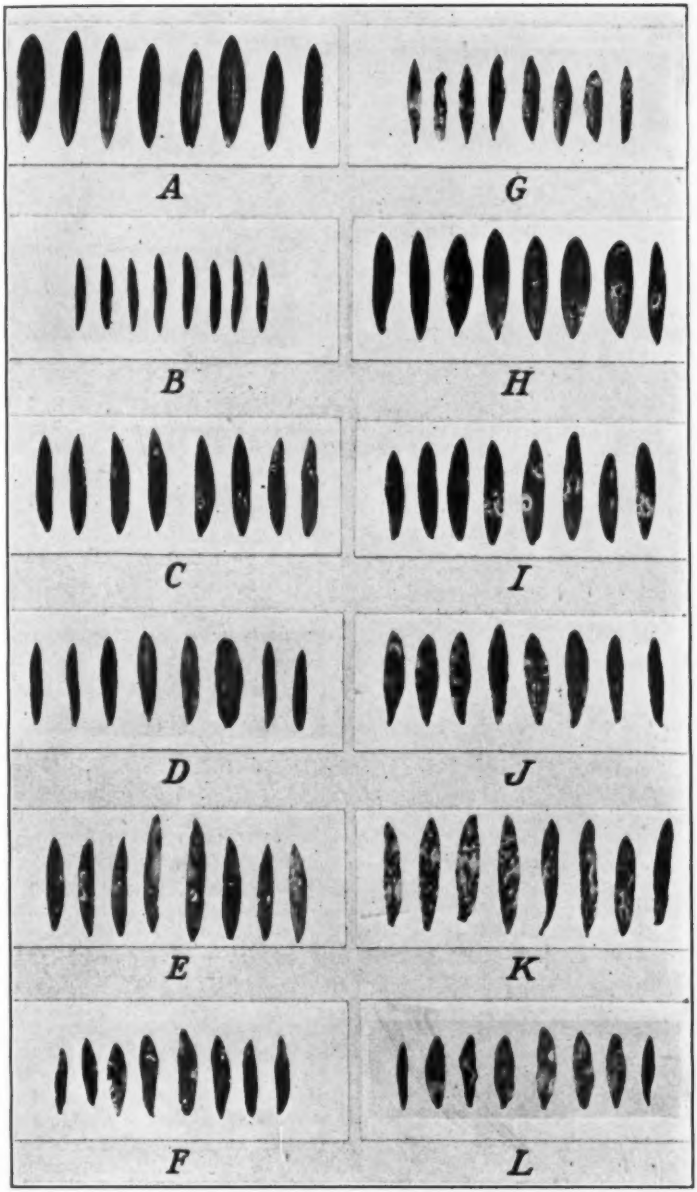
TABLE 1.—*Rust reaction of the parental varieties in the field in 1934 and 1935*

Variety	1934	1935	Variety	1934	1935
Ottawa 770B	Immune	Immune.	C. I. 649	Resistant	Resistant.
Newland	do	Do.	Bison	Semiresistant	Semiresistant.
C. I. 438	do	Do.	Redwing	do	Do.
Long × E	do	Do.	C. I. 391	Moderately susceptible.	Moderately susceptible.
C. I. 416–3	do	Do.			
C. I. 712	do	Do.	Pale Blue	do	Do.
Bolley Golden	[1]	Resistant or immune.	Abyssinian Yellow.	do	Do.
Light Mauve	Immune	Resistant.	C. I. 479	do	Do.

[1] Leaves destroyed; reaction could not be determined.

No rust was found in either year on the six varieties which were considered immune in outlining the experiment, i. e., Ottawa 770B, Newland, C. I. 438, Long × E, C. I. 416–3, and C. I. 712. In addition, all plants of Light Mauve were entirely free from evidence of rust infection in 1934, but in 1935 this variety showed flecks and small resistant pustules. Likewise, a small proportion of the plants of C. I. 649 grown in 1934 were free from rust, the remainder showing only a few resistant-type pustules per plant, while in 1935 occasional large pustules were found along with flecks and resistant pustules on this variety. In 1934, the leaves of Bolley Golden were entirely destroyed by the pasmo disease, making it impossible to determine rust reaction. In 1935 one plant row of Bolley Golden, from the plant used as a parent in the cross C. I. 479 × Bolley Golden, was immune, the remaining plants of Bolley Golden showing a resistant reaction.

Large susceptible-type pustules were found in Bison and Redwing, but in these two varieties the pustule frequency was less than in the four moderately susceptible varieties, C. I. 391, Pale Blue, Abyssinian Yellow, and C. I. 479. Thus the semiresistance of Bison and Redwing under these conditions was a matter of lower pustule frequency rather than type of reaction. Likewise the four moderately susceptible varieties differed from susceptible varieties such as Winona in having a lower percentage of rust rather than in type of pustule. The data on rust reaction in the F_2 generation of Ottawa 770B × Pale Blue and the F_2 and F_3 generations of Ottawa 770B × Redwing grown in the field in 1934 are summarized in table 2.



Classes of rust reaction used in classifying material in the greenhouse studies: *A*, Class 0; *B*, class 1; *C*, class 2 (pustules surrounded by necrosis); *D*, class 2 (pustules surrounded by chlorotic tissue) *E*, class 3; *F*, class 4; *G*, class 5; *H*, class 6; *I*, class 7; *J*, class 8; *K*, class 9; *L*, class 10.

TABLE 2.—*Segregation for rust reaction in the field of F_2 and F_3 generations of crosses involving Ottawa 770B as the immune parent*

Cross	Generation	Plants or lines showing indicated reaction		
		Immune	Segregating	Rusted
		Number	*Number*	*Number*
Ottawa 770B × Redwing	F_2 plants	44		13
Do	F_3 lines	31	47	21
Ottawa 770B × Pale Blue	F_2 plants	45		10

The rusted segregates in Ottawa 770B × Redwing all appeared semiresistant like Redwing. In the F_2 generation the number of plants was small but the fit to a 3:1 ratio was good, P lying between 0.70 and 0.80. The F_3 generation offers more critical evidence regarding the mode of inheritance of immunity. The fit of the observed ratio of F_3 lines to the theoretical 1:2:1 ratio was good, P lying between 0.30 and 0.50. χ^2 for fit to a 3:1 ratio was calculated for each of the 47 segregating F_3 lines separately and the χ^2 values added. The total χ^2 was 38.519. Applying the formula $\sqrt{2\chi^2}-\sqrt{2n-1}$ given by Fisher (*2*) a normal deviate of -0.866 ± 1 was obtained, the deviation from the expected 3:1 ratio not being significant. In the cross Ottawa 770B × Pale Blue, the number of F_2 plants was again too small to warrant drawing definite conclusions although the observed segregates approached the expected 3:1 ratio, with P lying between 0.20 and 0.30. These data are in agreement with the conclusion reached by Henry (*6*) that the immunity of Ottawa 770B in the field is conditioned by a single dominant factor.

GREENHOUSE STUDIES

The major portion of this paper is concerned with the reaction of the parents and hybrids under greenhouse conditions to a collection of rust and to physiologic race 4. It is known that in wheat certain varieties which are very susceptible to rust in the seedling stage in the greenhouse are highly resistant to the same forms of rust in the "mature-plant" stage in the field (Stakman (*8*), Hayes, Stakman, and Aamodt (*4*), and others). Flor (*3*) found that certain varieties of flax showing some resistance in the field were susceptible in the greenhouse. Therefore it seems desirable to know the rust reaction in the greenhouse of the varieties used as parents in this study before attempting to analyze the data obtained on the hybrids. Furthermore, the 11 classes of rust reaction used in classifying the parents and hybrids, were set up to include all of the differences which seemed sufficiently clear-cut to permit their classification, but no attempt was made at that time to determine their genetical significance. Such information, however, is valuable in analyzing the results obtained in segregating populations, and the best evidence of the validity or significance of the different rust classes from a genetical standpoint is to be found in the reaction of different biotypes under the conditions of the experiment.

PARENTAL REACTION

The data on the reaction of the parental varieties to a collection of rust and to form 4 are presented in table 3.

TABLE 3.—*Reaction of plants of parental checks to the collection of rust in the greenhouse and to form 4*

Variety	Plants in rust class—										
	0	1	2	3	4	5	6	7	8	9	10
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
REACTION TO COLLECTION OF RUST IN GREENHOUSE											
Ottawa 770B	451										
Newland	150										
C. I. 438	113										
Long × E	113	72	14								
C. I. 416–3	123	38									
C. I. 712	34	94	36								
Bolley Golden	66	65	86								
Light Mauve	38	72	94								
C. I. 649		21	15	73		30					
Bison				2	10	81	31	33			4
Redwing						48		55		18	1
C. I. 391						25		35			
Pale Blue							2	120		11	13
C. I. 479							5	45	1	47	38
Abyssinian Yellow								53			3
REACTION TO FORM 4											
Ottawa 770B	346										
Newland	145										
C. I. 438	156										
Long × E	123	32	1								
C. I. 416–3	130	35									
C. I. 712	30	80	32								
Bolley Golden	52	63	28								
Light Mauve	32	73	61								
C. I. 649		17	61	42		2					
Bison			5	45		157		2			
Redwing						2		131			
C. I. 391								46			
Pale Blue								181			
C. I. 479								109			
Abyssinian Yellow								67			

All the plants of Ottawa 770B, Newland, and C. I. 438 were immune, both from the collection and from form 4. These varieties had also been immune in the field. Two other varieties, Long × E and C. I. 416–3, immune under field conditions, showed a mixed reaction in the greenhouse.

Such variable or mixed reaction may either have been the result of genetical variability or the influence of environment. Since bulk seed of both these varieties was used in making the crosses, the possibility that the plants tested in each variety did not represent a single biotype cannot be excluded. Four plants of Long × E were used as parents in making the crosses in the greenhouse in 1933–34 and the offspring of one single plant selection from the progeny of each of these four plants were tested both to the collection and to form 4. The data are given in table 4.

TABLE 4.—*Reaction to collection of rust in greenhouse and to form 4 of offspring of single-plant selections from the progeny of plants of Long × E and C. I. 712 used as parents*

LONG × E

1933–34, plant no.	1934, no.	1935, no.	Plants in rust class indicated when inoculated with—						
			Collection				Form 4		
			0	1	2	3	0	1	2
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
132–1	4358–1	6107–2	16	2			12	4	
132–2	4367–1		5	12	3		1	12	1
133–4	4377–1	6189–1	5	7			30	7	
134–1	4373–1		20				14		
			13	3			12		

C. I. 712

140–1	4340–1	6005–1	5	7			3	12	
	4365–3		9	12			10	7	
140–4	4368–1			7		2		15	3
								14	4
141–1	4352–1					13		6	14
				3		16		9	11
142–2	4362–1			13		1			

The progeny of the two plants 132–1 and 134–1 were predominantly class 0, whereas the progeny of 132–2 were mostly class 1, both to the collection and to form 4. These results, although not conclusive, indicate that two different strains of Long × E had been used. This does not answer the question, however, whether the occurrence of both class 0 and class 1 progenies from the same plant resulted from heterozygosity for factors conditioning rust reaction or from the influence of environment. In a naturally self-pollinated crop like flax, the proportion of plants heterozygous for any given factor or factors, after the variety has been grown for several years, must be low. Therefore, it is unusual that all four of the plants used in the greenhouse should have been heterozygous and, in addition, that the single plant selected from their progeny should, in each of the four cases, also have been heterozygous.

Four plants of C. I. 416–3 were used as parents in the crosses made in the greenhouse. From the progeny of a single plant selected from the offspring of one of these original plants, three plant selections were made, the progeny of which were used as checks in the greenhouse rust tests. Likewise two plants from the progeny of a single plant selected from offspring of another original parent plant and single-plant selections from the progeny of the other two original parents were taken, the progeny of which were used as checks in the greenhouse tests. No sharp differences in the rust reaction of the different parental lines were observed. The greatest difference was between two pots planted with seed of a single plant. The 18 plants in one pot, inoculated October 13, were all class 0, while the 19 plants in the other pot, inoculated on November 25, were all class 1. These results indicate that in this variety the class 0 and class 1 types of reaction are subject to environmental fluctuations to a considerable extent.

The complexity of studies of disease resistance has been pointed out by other investigators. Reaction of the host to a disease is not merely the interaction of the genetic factors of a single organism with the environment but rather the interaction of two organisms, the host and the pathogen, each with its gene complex interacting with the environmental conditions. If immunity in flax is dependent upon the limitation of the development of the pathogen rather than its exclusion, it would perhaps not be surprising to find cases in which the balance between host and pathogen is so delicate that slight changes in the environment shift the reaction from one in which no macroscopic disease symptoms occur to one in which sufficient host cells are killed to produce a fleck.

C. I. 712, which was immune in the field, when tested in the greenhouse had plants in the three rust classes, 0, 1, and 2. Bulk seed of this variety had also been used for making the original crosses. Four plants were used as parents for the crosses made in 1933–34. The data on rust reaction of lines descending from these four plants are summarized in table 4.

Offspring from the plant selections from the progeny of 140–1 consist of plants in rust classes 0 and 1, while progeny of the other three lines have all plants in classes 1 and 2.

Two plants of Bolley Golden were used as parents in the greenhouse in 1933–34. Sixty-six plants of the line descendant from plant 123–2 were inoculated with the collection of rust in the greenhouse and all were immune. In addition, 32 plants of this line were inoculated with form 4 with the same results. This is the same line that was immune in the field. Progeny from four single-plant selections from offspring of the other parent plant varied in rust reaction from class 1 to class 2. Four pots were planted with seed from one of these single-plant selections and inoculated with the collection of rust. Plants in two of these pots fell in class 1, whereas those in the other two pots, inoculated at a later date, were classed as 2, i. e., with resistant-type pustules. This indicates that classes 1 and 2 may represent environmental fluctuations rather than true genetical differences, in this variety at least. In 1934, three plants of Bolley Golden were used in making additional crosses. Progeny of one of these plants were all class 0 while the progeny of the other two varied in rust reaction from class 1 to 2. It is probable that the variety Bolley Golden consists of a mixture of immune and resistant biotypes. Flor (*3*) found that 70 percent of the plants of Bolley Golden were immune, and 30 percent were resistant in his tests.

A similar situation obtained in Light Mauve. Thirty-nine individuals from a single-plant selection of the progeny of one of the parent plants used in making the crosses in the greenhouse were inoculated with the collection of rust. Thirty-six were class 0 and three were class 1. Eighteen plants of the same line were inoculated with form 4 and all were class 0. Progenies of plant selections from descendants of the other three original plants, when inoculated with the collection, were all classed as 1 or 2 except two, which were class 0. These two may have been escapes. When inoculated with form 4, all were placed in classes 1 and 2. No distinct differences in rust reaction between the descendants of these three original plants were observed. Three plants of Light Mauve were used in making additional crosses in 1934. Progenies of two of these, when tested with the collection of rust,

were classed as 1 and 2. Progenies of all three plants when tested with form 4 contained class 0, 1, and 2 plants. Whether the plants in class 0 represented true immunity or escapes is not known.

The plants of C. I. 649 varied from class 1 to class 5 both when inoculated with the collection and when inoculated with form 4. The proportion of class 1 plants was greater, and that of class 3 and 5 plants smaller, among the plants inoculated with form 4 than among those inoculated with the collection. The range in rust reaction from class 1 to class 5 is greater than would normally be expected in plants of the same genotype, particularly on such plants growing in the same pot. However, the seed of C. I. 649, used in making the greenhouse crosses, was taken from a single-plant selection, and the progeny of five single-plant selections from the offspring of two of the original parent plants gave, in each case, a similar range in reaction types. These results would suggest homozygosity of the material, although the only critical evidence would be a progeny test of individuals representing the four reaction classes. Such tests have not as yet been made. Marquillo wheat is known to show variability in reaction, giving both resistant and semiresistant plants (Hayes, Stakman, and Aamodt (*4*), Ausemus (*1*). Whether this is an analogous situation is not known.

The plants of Bison, inoculated with the collection of rust, varied from class 3 to 10, while the plants inoculated with form 4 were placed in classes 2, 3, 5, and 7. These results indicate that Bison is likewise more resistant to form 4 than to the collection. Flor (*3*) did not find such a difference either with C. I. 649 or Bison. Since, in the present study, the varieties were inoculated with form 4 and the collection at different times; it is not certain that a true difference in resistance obtains. Although the range in rust reaction among the Bison plants was large, a study of the progenies of the individual plant selections afforded no evidence regarding the nature of this variation. Classes 4 and 5 have a similar type of reaction and differ only in the pustule frequency. Class 4 was found in the early inoculations, but later all plants showing this type of pustule fell in class 5, perhaps due to a more uniform distribution of inoculum as the inoculation technique improved. A similar situation obtained for classes 6, 7, and 8, where the relative proportion of class 7 plants increased not only in Bison but also in other varieties to be discussed later, as the experiment progressed.

Plants of Redwing, inoculated with the collection, occurred in classes 5, 7, 9, and 10, while C. I. 391 had plants in classes 5 and 7. Class 5 plants were found in Redwing early in the experiment, while all plants inoculated later, under reduced intensity and duration of light, were classed as 7. In both of these varieties, when inoculated with form 4, all plants were placed in class 7 except two plants of Redwing in class 5.

Plants of Pale Blue and C. I. 479 varied from class 6 to class 10 when inoculated with the collection. In the early part of the investigation, the "green island" type of reaction of classes 9 and 10 occurred in the same pots of these two varieties along with classes 6, 7, and 8. Later, with reduced light, this type of reaction no longer appeared. All plants of these two varieties, when inoculated with form 4, gave a uniform class 7 type of reaction. It is doubtful from the results ob-

tained with these two varieties whether classes 6, 7, 8, 9, and 10 represent true genetical differences.

On the basis of the above results, it appeared logical to place the parents in five groups, as follows:

(1) Immune: Ottawa 770B, Newland, C. I. 438, and the immune strain of Bolley Golden.
(2) Near immune: Long × E, C. I. 416–3, and the near-immune strain of Light Mauve.
(3) Resistant: Bolley Golden, Light Mauve, and C. I. 712.
(4) Semiresistant: Bison, Redwing, and C. I. 391.
(5) Susceptible: Pale Blue, C. I. 479, and Abyssinian Yellow.

C. I. 649 is less resistant than Bolley Golden, Light Mauve, or C. I. 712. On the other hand, it is definitely more resistant than the varieties classed as semiresistant. Because of the variable reaction of the plants of C. I. 649 in this study, this variety has not been placed in any of the five groups.

C. I. 649 was used in crosses with one immune variety, Ottawa 770B, one resistant variety, Light Mauve, and three semiresistant varieties, Bison, Redwing, and C. I. 391.

On the basis of the rust reaction of the parents under greenhouse conditions, the crosses can be classified as those involving parents with similar rust reaction and those involving parents with different rust reaction, as follows:

Crosses involving parents with similar rust reactions:

Immune × immune: Ottawa 770B × Newland, Ottawa 770B × C. I. 438, and C. I. 438 × Newland.
Near immune × near immune: C. I. 416–3 × (Long × E).
Semiresistant × semiresistant: Redwing × Bison.
Susceptible × susceptible: C. I. 479 × Pale Blue and Pale Blue × Abyssinian Yellow.

Crosses involving varieties with different reaction to rust:

Immune × near immune: Ottawa 770B × C. I. 416–3, Ottawa 770B × (Long × E), C. I. 416–3 × Newland, C. I. 416–3 × C. I. 438, C. I. 438 × (Long × E), and (Long × E) × Newland.
Immune × resistant: Ottawa 770B × Bolley Golden, Ottawa 770B × Light Mauve, Ottawa 770B × C. I. 712, C. I. 712 × Newland, and C. I. 712 × C. I. 438.
Immune × semiresistant: Ottawa 770B × Redwing and Ottawa 770B × Bison.
Immune × susceptible: Ottawa 770B × Pale Blue, Ottawa 770B × C. I. 479, and C. I. 479 × Bolley Golden.
Near immune × resistant: C. I. 712 × (Long × E) and C. I. 712 × C. I. 416–3.
Near immune × semiresistant: Light Mauve × Redwing.
Resistant × semiresistant: Light Mauve × Bison and C. I. 391 × Light Mauve.
Resistant × susceptible: Bolley Golden × Pale Blue and Bolley Golden × Abyssinian Yellow.
Semiresistant × susceptible: Pale Blue × Bison and C. I. 479 × Bison.

REACTION OF HYBRID MATERIAL

Immune × Immune

In the cross of Ottawa 770B × C. I. 438, 32 F_3 lines, averaging 15.6 plants per line, were inoculated with the collection of rust and only class 0 plants were obtained. Inoculation of these same F_3 lines with form 4 gave identical results. The results indicate that the immunity of Ottawa 770B is allelic to the immunity of C. I. 438.

The data on the reaction to the collection of rust of parents, F_1, and F_2 of Ottawa 770B × Newland and the parents and F_2 generation of C. I. 438 × Newland are presented in table 5.

TABLE 5.—*Reaction of parents, and of F_1 and F_2 generations of Ottawa 770B × Newland and C. I. 438 × Newland to collection of rust in greenhouse*

Parent or generation	Number of plants or lines showing indicated reaction [1]						
	I	I, R	I, SR	I, SR, S	I, S	R	S
Ottawa 770B---plants	34						
Newland---do	20						
F_1---do	5						
F_2---lines	20		1	2	11		1
C. I. 438---plants	31						
Newland---do	32						
F_2---lines	16	13				1	

[1] I=immune, R=resistant, SR=semiresistant, S=susceptible.

In the 35 F_2 lines of Ottawa 770B × Newland, semiresistant (class 5) and susceptible segregates were obtained in addition to class 0 plants. Grouping the class 5 and susceptible segregates together, the ratio of F_2 lines becomes 20 immune : 14 segregating : 1 susceptible. Using χ^2 to compare this ratio with the calculated, on the basis of duplicate factors for immunity, gave a value of P between 0.10 and 0.20. These data suggest the hypothesis of a single dominant factor conditioning immunity in each variety the two factors not being allelic and inherited independently.

The relationship of the reaction of these lines to the collection and to form 4 is shown in table 6.

TABLE 6.—*Correlation table showing the reaction of F_2 lines of Ottawa 770B × Newland to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection			
	Immune	Segregating	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Immune	20	5		25
Segregating		8		8
Susceptible		1	1	2
Total	20	14	1	35

From these data it can be seen that the reaction of the F_2 lines to form 4 was similar to their reaction when inoculated with the collection. Five lines that were classed as segregating with the collection contained only class 0 plants when inoculated with form 4. However, since the average number of plants per line was only 11.5 in the test with form 4, these exceptions probably resulted from failure to recover recessive segregates in some lines owing to the small number of plants. In addition, one line was classed as segregating with the collection and susceptible to form 4. In this line only three plants were tested in each case.

In C. I. 438 × Newland (table 5), 16 of the F_3 lines contained only class 0 plants, 13 contained, in addition to class 0, class 1, 2, or 3 segregates and 1 line contained only class 1, 2, and 3 plants. The occurrence of class 1, 2, and 3 plants in these F_3 lines suggests the absence of allelism of the factor for immunity in Newland with a factor conditioning immunity in C. I. 438. χ^2 for fit of observed to calculated on the basis of two dominant factors for immunity gave a P value between 0.20 and 0.30. Since no susceptible segregates were obtained in the F_3 lines, it appears that one of the varieties carries, in addition to a factor for immunity, a factor for resistance allelic to the factor conditioning immunity in the other variety. No evidence of a factor determining resistance was found in the cross of Ottawa 770B × Newland. Therefore, it seems probable that C. I. 438 carries at least two factors, one conditioning immunity and the other, which is allelic to the factor for immunity in Newland, conditioning resistance to the collection of rust. When the F_3 lines of C. I. 438 × Newland were inoculated with form 4, all plants were immune. This indicates that the factor in C. I. 438 which conditions resistance to the collection determines immunity from form 4.

Near Immune × Near Immune

In C. I. 416–3 × (Long × E), 25 F_3 lines, averaging 12.7 plants per line, were tested with the collection of rust. Only class 0, 1, and 2 plants were obtained. The variability of the two parents from class 0 to 1 made an exact genetical interpretation of the results in the F_3 lines impossible. However, the absence of semiresistant or susceptible segregates in any of the F_3 families indicates that the near immunity of these two varieties are allelic. Likewise, only class 0, 1, and 2 plants were obtained when these lines were inoculated with form 4.

Semiresistant × Semiresistant

The data on the reaction of parents, F_1, and F_3 generations of Redwing × Bison to the collection and to form 4 are given in table 7.

Of the 32 F_3 lines inoculated with the collection, 14 contained only class 4 and 5 plants, while the remaining 18 contained both class 4 and 5 and susceptible segregates. The reaction of Bison and Redwing is variable, as pointed out in the analysis of the parental reaction in the greenhouse, thus precluding the formulation of any factorial explanation of the results obtained in this cross.

The results with form 4 differ somewhat from those obtained with the collection in this cross. One of the plants of Redwing was placed in class 5 and 28 in class 7, whereas the Bison plants were placed in classes 3, 5, and 7. Here again a factorial explanation of the results in F_3 cannot be given. It is interesting to note that the proportion of class 3 segregates in the material inoculated with form 4 is greater in those lines showing only class 4 and 5 segregates when inoculated with the collection. Likewise, the proportion of plants showing a susceptible reaction to form 4 is greater in those lines showing susceptible segregates with the collection of rust.

TABLE 7.—*Reaction of parents, and of F_1 and F_3 generations of Redwing × Bison to collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—									
	Collection							Form 4		
	4	5	6	7	8	9	10	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Redwing		30					1		1	28
Bison		32						5	29	1
F_1		5							5	
F_3 families:										
6011-7		16						5	7	
6011-13		17							11	4
6011-15		15							10	6
6011-16		15						1	14	
6011-20		20							13	4
6011-21		14							9	4
6011-27		3						2	6	6
6011-28		20						3	14	
6011-31		16						10		
6011-8	3	9						2	7	
6011-10	9	4						2	7	5
6011-12	1	16						6	8	
6011-17	1	17							13	
6011-30	4	12						4	10	
6011-5	4	10		2				2	8	4
6011-2		6					6			16
6011-3		9					3	1	7	7
6011-4		8					6			10
6011-6		16					3			14
6011-19		12					3		3	8
6011-22		11					1	2	9	5
6011-23		17					1	1	14	
6011-24		14					2		13	5
6011-25		9					6	1	10	6
6011-11		8		3			6		12	5
6011-18		9		1			3		6	10
6011-29		5		4			9		1	17
6011-9		4				3	6		10	5
6011-32		11				1	3		12	4
6011-1		9		1		3	1	1	7	5
6011-14		2			1	11	1			18
6011-26		4	1	2	1		3		11	

SUSCEPTIBLE × SUSCEPTIBLE

In the cross Pale Blue × Abyssinian Yellow, all plants of both parents and the F_1 were classed as susceptible in the material inoculated with the collection, while in the 30 F_3 lines, 3 contained only class 5 plants, 19 contained class 5 and susceptible plants, and 8 contained only susceptible segregates. On the basis of the results obtained in Bison, Redwing, and C. I. 391, the varieties showing some semiresistant plants, it is evident that the class 4 and 5 type of reaction is not very sharply differentiated genetically from susceptible. Consequently, it is possible that the class 5 plants obtained among the progeny of this cross were merely fluctuations caused by environment. However, the absence of such plants in either parental variety suggests a true genetical difference, although it is impossible to give a factional explanation of the results. When this cross was inoculated with form 4, all plants of the parents, F_1 and F_3 lines were susceptible.

In the cross C. I. 479 × Pale Blue, all plants of the parents, F_1, and of 63 F_3 lines were susceptible both to the collection and to form 4.

Immune × Near Immune

The data on the reaction of the parents, F_1, and F_3 lines of Ottawa 770B × C. I. 416–3 are presented in table 8.

Table 8.—*Reaction of parents, and of F_1 and F_3 generations of Ottawa 770B × 416–3 to collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—									
	Collection					Form 4				
	0	1	2	3	7	0	1	2	3	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Ottawa 770B	21					39				
C. I. 416–3		34				34	6			
F_1	1									
F_3 lines:										
4212–4	17					19				
4212–12	18					19				
4212–22	20					16				
4212–27	20					20				
4212–33	20					19				
4212–37	10					19				
4212–2	15	4				18				
4212–6	12	5				15	1			1
4212–10	19	1				14				
4212–11	12	3				18				1
4212–14	8	3				18				
4212–19	15	2				17				
4212–23	17	3				16	3			
4212–24	15	3				10	4			
4212–28	16	4				18				
4212–30	18	2				17	2			
4212–32	15	2				16	1	1		
4212–36	10	4								
4212–15	9	6				16				
4212–21	5	12	1			17	1			
4212–7	17				2	13	1	1		2
4212–13	9				3	17				
4212–18	16				4	13	7			1
4212–38	11				3	13		2		2
4212–11	12	3			1	18				1
4212–29	14	1		2	3	16				3
4212–3	1	17			2		16			3
4212–25		16	2	2		14	2			
4212–31		17	1	1		10	4	1		4
4212 8		20				18	2			
4212–17	1			2	15					20
4212–26				5	13	1		9	4	6

In the material inoculated with the collection, 2 of the 32 F_3 lines were predominantly susceptible although 2 plants out of the 18 in 1 line and 5 out of 18 in the other were placed in class 3. The single class 0 plant in one of these two lines may have been a natural hybrid. The occurrence of two susceptible lines and of susceptible segregates in seven other lines indicates that the near immunity of C. I. 416–3 is not allelic to the immunity of Ottawa 770B. Although the number of lines showing susceptible segregates was less than would be expected, the results in this cross could be explained on the hypothesis of two dominant factors, one conditioning the immunity of Ottawa 770B and the other determining the near immunity of C. I. 416–3. However, since the mean number of plants in the F_3 lines was 17.56, the numbers are too small to warrant definite conclusions regarding the number of factors involved.

TABLE 9.—*Correlation table showing the reaction of F_3 lines of Ottawa 770B × C. I. 416–3 to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection			
	Immune, near immune	Segregating	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Immune, near immune	19	1		20
Segregating	3	6	1	10
Susceptible			1	1
Total	22	7	2	31

The relation of the reaction of the F_3 lines to the collection and their reaction to form 4 is shown in table 9. Since classes 0, 1, 2, and 3 do not appear to be differentiated by major genetic factors in this cross, these four classes are combined in this case. The results indicate that the major factors differentiating the reaction of the F_3 lines to the collection of rust are the same as those conditioning reaction to form 4.

In the material of Ottawa 770B × (Long × E), inoculated with the collection, 31 F_3 lines, averaging 15 plants per line, were studied and only class 0, 1, and 2 segregates were obtained, indicating that the near immunity of Long × E is allelic to the immunity of Ottawa 770B. The variability of the near immune reaction of Long × E made an exact factorial explanation of the results in this cross impossible. When these F_3 lines were inoculated with form 4, only class 0, 1, and 2 plants were again obtained.

In the 35 F_3 lines of (Long × E) × Newland, no semiresistant or susceptible segregates were obtained either in the material inoculated with the collection or with form 4, indicating that the factor conditioning immunity in Newland is allelic to a factor for near immunity in Long × E. A similar result was obtained in inoculations both with the collection and the single form of 31 F_3 lines of C. I. 416–3 × Newland, likewise suggesting allelism of the near immunity of C. I. 416–3 with the immunity of Newland.

The data on the reaction of parents and F_3 families of C. I. 438 × (Long × E) are presented in table 10.

In the material tested with the collection, the occurrence of 6 F_3 lines out of 33 with class 5 and susceptible segregates suggests the possibility of genetical segregation. It is interesting to note that these segregates all occur in the progeny of one F_2 family, 5163. These results indicate that the two F_1 plants from which F_2 families 5163 and 6166 descended were not alike genetically. This perhaps resulted from the use of a heterozygous parent in making the cross. As can be seen in table 10, the behavior of the F_3 lines when tested with form 4 was similar to their reaction to the collection.

Of the 32 F_3 families of C. I. 416–3 × C. I. 438 inoculated with the collection of rust, all the plants in 31 lines were class 0, and in the other family 10 plants were class 0 and 1 plant was class 1. Also, all plants of C. I. 416–3, inoculated as checks with this cross, were class 0. The average number of plants in the F_3 lines in this cross was 12.75. When inoculated with form 4, all plants of C. I. 416–3 and of the

32 F_3 families were class 0. From these results it appears that the near immunity of C. I. 416–3 is allelic to the immunity of C. I. 438.

TABLE 10.—*Reaction of parents and of F_3 generation of C. I. 438 × (Long × E) to collection of rust in greenhouse and to form 4*

Parents or generation	Plants in rust class indicated when inoculated with—								
	Collection						Form 4		
	0	1	2	3	5	7	0	1	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
C. I. 438	39						39		
(Long×E)	33	3					26		
F_3 Families:									
6166–4	12						1		
6166–7	24						9		
6166–9	34						16		
6166–10	31						11		
6166–12	19								
6166–14	18						7		
6166–16	22						4		
6166–17	28						7		
6166–19	16						1		
5163–4	12						7		
5163–11	19						3		
5163–13	15						2		
6166–6	35	1					11		
6166–8	21	4							
6166–13	23	1					12		
6166–1	14	2							
6166–3	21	1					16	1	
6166–5	16	1					8	2	
6166–20	19	3					7		
5163–12	12	1					7		
5163–14	18	2					7		
5163–19	26	1					8		
5163–20	29	3					8		
6166–11	21	3	1				17		
6166–18	25	5	4						
6166–21	29	5	1				4	1	
5163–6	12			2			11		1
5163–8	17	1			1		4		1
5163–16	27		1		3		11		1
5163–17	15	3			1	1	13		
5163–18	25	3			1	1	10		
5163–22	4	4			3	1	5		2
5163–2	15	1				4	7	2	

IMMUNE × RESISTANT

In the crosses involving Ottawa 770B with Bolley Golden and Light Mauve, only F_2 generations were studied; the data for these crosses are presented in table 11.

TABLE 11.—*Reaction of parents and F_2 generation of crosses of immune × resistant varieties to collection of rust in greenhouse*

Parent or generation	Plants showing indicated reaction						
	0	1	2	3	5	7	9
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Ottawa 770B	75						
Bolley Golden		36	37				
F_2	448	32	70		4	24	4
Ottawa 770B	38						
Ligh Mauve	2	33	5				
F_2	169	2	8	3	5		1
Ottawa 770B	37						
Light Mauve		4	33				
F_2	294	1	51	6	5	16	

The plants of Bolley Golden occurred in the two classes 1 and 2. As pointed out in the discussion of parental reaction in the greenhouse, it seems probable that these two classes represent fluctuations due to environment rather than actual genetical differences in this variety. Likewise, the data on parental reaction indicated that classes 6, 7, 8, 9, and 10 did not represent true genetical differences, but rather variations due to environment. Consequently, the analysis of the results in segregating populations would be facilitated by combining class 1 and 2 into a resistant group and the last five classes into a susceptible group.

On this basis, the F_2 plants of Ottawa 770B $\times$ Bolley Golden occur in the ratio of 448 immune:102 resistant:4 class 5:28 susceptible. Considering the class 5 and susceptible segregates together, χ^2 for fit of the observed ratio to the theoretical 12:3:1 ratio gave a value of P between 0.50 and 0.70. Such a result could be explained on the assumption of a single dominant factor conditioning the immunity of Ottawa 770B and a single dominant factor for resistance carried by Bolley Golden which is not allelic but hypostatic to the factor for immunity contributed by Ottawa 770B. The validity of combining the four class 5 segregates with the susceptible group may be subject to question. However, these plants are definitely more susceptible than the resistant group of segregates, which resemble Bolley Golden in their rust reaction.

In Ottawa 770B $\times$ Light Mauve, a ratio of 169 immune:10 resistant:3 class 3:5 class 5:1 susceptible was obtained. Class 3 apparently represents a type of resistance and differs from class 2 of the resistant group only in having occasional large pustules in addition to the flecks and resistant type pustules. Combining the class 3 plants with the resistant group and again considering the class 5 and susceptible segregates together, a ratio of 169:13:6 obtains. χ^2 for fit of this ratio to a 12:3:1 ratio is 22.418, a value greatly in excess of χ^2 for the 1 percent point. The deviation from the expected ratio results from an excess of immune segregates and a deficiency particularly in the resistant class. Since, in this case, 2 of the 40 Light Mauve plants were classed as immune, the excess of class 0 segregates may have been due to escapes. To test this possibility, additional F_2 plants of this cross were inoculated. In the second trial, a ratio of 294 immune:52 resistant:6 class 3:5 class 5:16 susceptible segregates obtained. Again combining class 3 with the resistant group and class 5 with the susceptible group, χ^2 for fit of observed to a 12:3:1 ratio gave a value of P between 0.20 and 0.30. These results indicate that Light Mauve carries a single dominant factor for resistance which is not allelic but hypostatic to the factor conditioning immunity in Ottawa 770B.

In Ottawa 770B $\times$ C. I. 712, the F_1 plants were all immune both when tested with the collection and with form 4. The results in the 30 F_3 lines are summarized in table 12. In this case the class 0 plants were considered immune and the class 1, 2, and 3 segregates were again combined into a resistant group. Considering the resistant and semiresistant segregates together in the material inoculated with the collection, the observed ratio of 7 immune:14 segregating:9 not immune lines compared with the theoretical 1:2:1 ratio by means of χ^2, gave a value of P between 0.80 and 0.90. The reaction of the

F_3 lines to form 4 was similar to their reaction to the collection. However, two lines which showed a resistant reaction to the collection had mostly class 0 plants when inoculated with form 4. In addition, one line was predominantly class 0 with the collection, but when tested with form 4, seven of the eight plants were in class 1. It seems probable that these exceptions may be the result of environmental fluctuations rather than true genetical differences. Semiresistant segregates in two lines both when inoculated with the collection and with form 4, and the susceptible segregates in two lines when tested with form 4, may have resulted from the lack of allelism of the factors for resistance of C. I. 712 and the factor for immunity in Ottawa 770B or from the segregation of minor modifying factors. No evidence was available in this cross for determining which alternative is correct.

TABLE 12.—*Correlation table showing the reaction*[1] *of F_3 lines of Ottawa 770 B × C. I. 712 to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection—				
	I	I, R	I, SR	R	Total
	Number	*Number*	*Number*	*Number*	*Number*
I	6	5			11
I, R	1	6		2	9
I, SR			1		1
I, SR, S			1		1
I, R, S		1			1
R				7	7
Total	7	12	2	9	30

[1] See footnote 1, table 5.

Only class 0, 1, and 2, and 3 segregates were obtained in the 29 F_3 lines of C. I. 712 × C. I. 438 when inoculated with the collection. Combining classes 1, 2, and 3 into a resistant group, the ratio of F_3 lines was 10 immune:13 segregating:6 resistant. χ^2 for fit of this ratio to a 1:2:1 gave a value of P between 0.30 and 0.50. Seed was available for testing with form 4 of only 26 of the F_3 lines of this cross, and, of these, 14 contained only class 0 plants, 11 contained predominantly class 0 with class 1, 2, or 3 segregates in addition, and 1 line contained predominantly class 1 and 2 plants with 1 class 0 plant. This single line seemed to resemble C. I. 712 in reaction to rust. These results are similar to those obtained with C. I. 438 × Newland, and may be explained by assuming that C. I. 438 carries at least two factors for rust reaction, one conditioning immunity from both the collection and form 4, the other conditioning resistance to the collection and immunity from form 4.

The data on C. I. 712 × Newland are presented in table 13.

Of the 31 F_3 families of this cross, 18 contained susceptible or class 5 segregates, or both susceptible and class 5 plants when tested with the collection. The occurrence of these class 5 and susceptible segregates indicates that the resistance of C. I. 712 is not allelic to the immunity of Newland. Further, the proportion of lines showing sus-

ceptible segregates suggests that not more than two pairs of factors were involved. Considering the class 1, 2, 3, 5, and the susceptible plants together, the ratio of F_3 lines was 7 immune: 14 segregating : 10 not immune. Two of the lines classed as not immune each contained a single class 0 plant, but, since immunity is dominant, it is improbable that these plants resulted from genetical segregation. The fit of this observed ratio to a 1:2:1 is good, P lying between 0.50 and 0.70. Total χ^2 for goodness of fit to a 3:1 ratio within the segregating F_3 lines gave P between 0.30 and 0.50. These results are in agreement with the assumption of a single dominant factor conditioning the immunity of Newland. The reaction of the F_3 lines to form 4 seems, in general, to be similar to their reaction to the collection, particularly when the small number of plants per line is considered.

TABLE 13.—*Reaction of parents and of F_1 and F_3 generations of C. I. 712 × Newland to collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—											
	Collection						Form 4					
	0	1	2	3	5	7	0	1	2	3	5	7
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Newland	32						38					
C. I. 712		18	3									
F_1	2											
F_3 families:												
6154–1	9						1					
6154–6	4											
6154–8	13						6					
6154–9	20						19					
6154–14	11						10					
5151–3	7						3	1				
5151–9	9						4					
6154–4	6				2		11	1		2	1	1
6154–5	8					3	1		1			
6154–2	7		1	3			10					
6154–22	5		1	1			7		4		3	
6154–3	6	1				1	9	1		1	1	
6154–10	5	1				1	7		1	1		
6154–12	12	1				3	12				4	
6154–17	5		1			2						
5151–11	7	2				5	6					
5151–16	8			1		2	4			1	1	
5151–6	3				3	1	1				1	
6154–7	11		2	1		1	6					
6154–16	8	1		1		1						
6154–13	6			1	3	3	1		2			
6154–11		6	1						2			
6154–23		7					11	1	2			
5151–14		4	2					2	4			
6154–15	1		3	1								
6154–19		5	4	4		1		2	1	4	5	1
6154–20		1	3	3	6	5		3	4	2	4	
5151–2		13		1	3	1	3	2	6	3		
5151–5		2	1	2	2	1				4		
5151–8	1	2	1		4	2			1	1	5	
5151–10				5	1		2		2			

IMMUNE × SEMIRESISTANT

In the cross Ottawa 770B × Redwing the two F_1 plants were immune like Ottawa 770B, while the plants of Redwing were placed in classes 5 and 9. The data on the reaction of 63 F_3 lines to the collection and to form 4 are summarized in table 14.

TABLE 14.—*Correlation table showing the reaction*[1] *of F_3 lines of Ottawa 770B × Redwing to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection—						
	I	I, SR, S	I, SR	IS	SR, S	S	Total
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
I	15			1			16
I, SR, S		4		2			6
I, S		7	1	19			27
SR, S					2		2
S					7	5	12
Total	15	11	1	22	9	5	63

[1] See footnote 1, table 5.

The variability of the Redwing parent, in which both semiresistant (class 5) and susceptible plants occurred, illustrates the difficulty of formulating a genetical explanation of the occurrence of the semiresistant segregates. Considering the semiresistant and susceptible segregates together in the material inoculated with the collection, the ratio of the 63 F_3 lines was 15 immune: 34 segregating: 14 not immune. A comparison of this ratio with a 1:2:1 ratio by means of χ^2 gave a P value of 0.80 to 0.90. Total χ^2 for fit to a 3:1 ratio within the segregating F_3 families was 25.171 with 34 degrees of freedom. The normal deviate was -1.09 ± 1, indicating that the deviation from the expected within the segregating F_3 families was within the errors of random sampling.

The principal differences between the reaction to the collection and to form 4 of these 63 F_3 lines were found in the semiresistant and susceptible classes. Since the class 4 and 5 type of reaction was variable these differences may have represented variations due to environment. Considering the immune as contrasted to the not immune segregates, the agreement of reaction to the collection and to form 4 was nearly complete. One line which was segregating with the collection was classed as immune from form 4. These results indicate that the factor conditioning immunity from the collection likewise conditions immunity from form 4.

The data on the reaction of the F_3 families of Ottawa 770B × Bison are given in table 15.

No check plants of either parent inoculated at the same time as the hybrid material were available in studying this cross. Immune, class 5, and susceptible segregates occurred in the 30 F_3 lines when inoculated with the collection. Here again it is impossible to offer a factorial explanation for the occurrence of class 5 segregates. Combining the class 5 and susceptible segregates, the ratio of F_3 lines was 10 immune : 9 segregating : 11 not immune. χ^2 for fit to a 1 : 2 : 1 ratio gave a value of P between 0.05 and .010. Total χ^2 for fit to a 3 : 1 ratio within the segregating F_3 lines gave a P value between 0.70 and 0.80, a good fit of observed to calculated. The reaction of the F_3 lines of this cross to form 4 indicates that the factor conditioning immunity from the collection likewise conditions immunity from form 4. When these F_3 lines are inoculated with form 4, class 2 and a number of class 3 segregates occurred. Likewise, Bison, in other tests, contained

some class 2 and class 3 plants when inoculated with form 4. No factorial explanation of these results seems possible.

TABLE 15.—*Reaction of F_3 families of Ottawa 770B × Bison to a collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—									
	Collection					Form 4				
	0	5	7	8	9	0	2	3	5	7
F_3 families:	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
6148–1	16					15				
6148–4	18					13				
6148–5	18					19				
6148–6	6					14				
6148–9	14					17				
6148–16	17					12				1
6148–22	3					4				
6148–24	11					12				
5145–1	9					8				
5145–2	17					19				1
5145–3	7	1				4				4
6148–3	16			5		15		1	1	
6148–7	13		2	2		10		3	2	1
6148–8	13			2		16		2		1
6148–11	13			6		12				6
6148–18	13			4		12		1	3	1
6148–20	3			2		3			1	
5145–12	7		1			2	3			
5145–17	7			6		8			3	2
6148–2	1	7		10	1		3	17		
6148–17		1		5			2	1	11	4
6148–21		2		3					2	1
6148–23		1	7	10					2	9
5145–4		2		4				3	4	1
5145–7		6	1	5					9	4
5145–15		4		11		1		5	6	
6148–12				15						17
5145–5				7						11
5145–10			6	1				1	5	
5145–11		3					1	1		

IMMUNE × SUSCEPTIBLE

In Ottawa 770B × Pale Blue and Ottawa 770B × C. I. 479, the not immune segregates varied from class 6 to class 10. Since, as already pointed out, these five classes probably represent only fluctuations due to environment, they have been combined in the genetical analysis of these crosses into a susceptible group. The data on these two crosses when inoculated with the collection of rust are summarized in table 16.

TABLE 16.—*Reaction of parents, and of F_1 and F_3 families of Ottawa 770B × Pale Blue and Ottawa 770B × C. I. 479 to collection of rust in greenhouse*

Parent or generation		Plants or lines showing indicated reaction		
		Immune	Segregating	Susceptible
Ottawa 770B	plants	33		
Pale Blue	do			38
F_1	do	5		
F_3	lines	11	33	10
Ottawa 770B	plants	38		
C. I. 479	do			30
F_3	lines	13	34	17

In Ottawa 770B × Pale Blue, the F_1 was immune like Ottawa 770B. The fit of the F_3 lines to a 1 : 2 : 1 ratio was good, P lying between 0.30 and 0.50. Total χ^2 for fit to a 3 : 1 ratio within the segregating F_3 lines was 33.788 with 33 degrees of freedom. The normal deviate of 0.16 ± 1 was clearly not statistically significant. Likewise, in Ottawa 770B × C. I. 479, the fit of the F_3 lines to the theoretical 1 : 2 : 1 ratio was good, P lying between 0.50 and 0.70. Total χ^2 for fit to a 3 : 1 ratio within the segregating F_3 lines was 26.618 and the normal deviate was −0.889 ± 1. Thus the deviation from the expected ratio was again within the errors of random sampling. These results strongly support the hypothesis of a single dominant factor determining the immunity of Ottawa 770B. The relation of the reaction of the F_3 lines of Ottawa 770B × Pale Blue when inoculated with the collection to their reaction with form 4 is shown in table 17. The agreement of reaction in this cross is complete. A

TABLE 17.—*Correlation table showing the reaction of F_3 lines of Ottawa 770B × Pale Blue and Ottawa 770B × C. I. 479 to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection			
	Immune	Segregating	Susceptible	Total
OTTAWA 770B × PALE BLUE				
	Number	*Number*	*Number*	*Number*
Immune	11			11
Segregating		33		33
Susceptible			10	10
Total	11	33	10	54
770B × C. I. 479				
Immune	12	1		13
Segregating	1	33		34
Susceptible			17	17
Total	13	34	17	64

similar comparison for Ottawa 770B × C. I. 479 is also shown in table 17. In this cross the agreement is again complete except for two lines. These two exceptions have probably resulted from the chance failure to recover recessive segregates among the progeny of heterozygous F_2 plants. It seems probable that, in these crosses, the factor for immunity from the collection of rust likewise conditions immunity from form 4.

The data on the reaction to the collection of rust or the parents and F_2 generation of C. I. 479 × Bolley Golden are presented in table 18

TABLE 18.—*Reaction of parents and F_2 generation of C. I. 479 × Bolley Golden to collection of rust in greenhouse*

Parent or genaration	Plants showing indicated reaction					
	0	1	2	7	9	10
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Bolley Golden	65					
C. I. 479				20	35	5
F_2	246	38	27	13	3	8

Combining classes 1 and 2 into a resistant group and classes 7, 9, and 10 into a susceptible group, the F_2 ratio was 246 immune : 65 resistant : 24 susceptible. χ^2 for fit of this ratio to an expected ratio of 12:3:1 gave a value of P between 0.70 and 0.80. The results in the 55 F_3 lines are summarized in table 19.

TABLE 19.—*F_3 lines of C. I. 479 × Bolley Golden, grouped according to their breeding behavior when tested with a collection of rust in greenhouse, and the expected ratio on the basis of two factor pairs, one for immunity and one for resistance*

Item	Lines showing indicated reaction [1]						
	I	IR, S	I, R	I, S	R	R, S	S
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Observed	12	7	15	10	5	4	2
Calculated	13.75	13.75	6.875	6.875	3.4375	6.875	3.4375

[1] See footnote 1, table 5.

Combining the last three classes because of the small numbers, χ^2 for goodness of fit was 15.109, a value in excess of χ^2 for the 1-percent point. The greatest deviation of observed from calculated occurs in the F_3 lines segregating for immune, resistant, and susceptible and in those segregating for immune and resistant. In the lines segregating for immune, resistant, and susceptible, on the assumption of two pairs of factors, only one-sixteenth of the plants in the line should be susceptible. Since the average number of plants per F_3 line was only 10.02, susceptible segregates would frequently not be obtained among the progeny of F_2 plants heterozygous for both pairs of factors, in which case the line would be incorrectly classed as segregating for immune and resistant. Since the numbers are too small to permit the accurate differentiation of these two classes, it seems logical to combine them in the analysis. χ^2 for comparing the observed and calculated ratios after combining these two classes, and also combining the last three classes in table 19, gave a value of P between 0.50 and 0.70. These results indicate that the immune strain of Bolley Golden carries a dominant factor conditioning immunity and a dominant factor for resistance to the collection of rust, the factor for immunity being epistatic to the factor for resistance.

TABLE 20.—*Correlation table showing the reaction [1] of F_3 lines of C.I. 479 × Bolley Golden to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection							
	I	I, R, S	I, R	I, S	R	R, S	S	Total
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
I	10		3		1			14
I, R, S		7		1				8
I, R	1		8	1				10
I, S	1		1	6				8
R					4			4
R, S						4		4
S							2	2
Total	12	7	12	8	5	4	2	50

[1] See footnote 1, table 5.

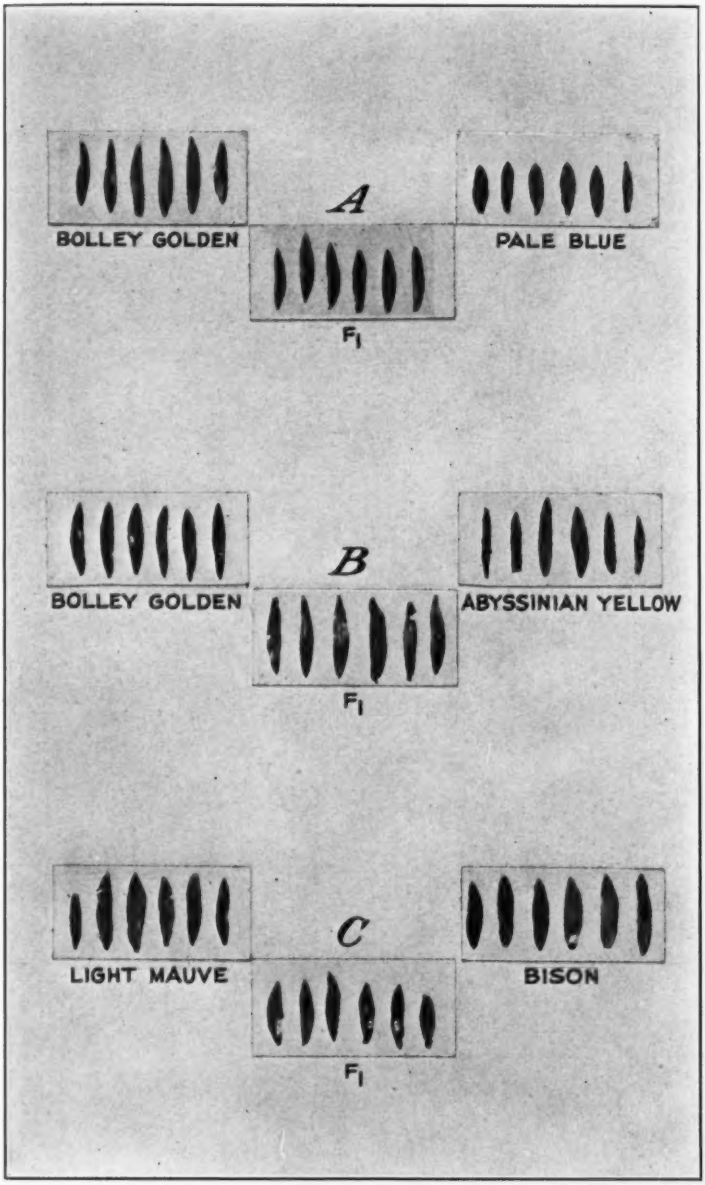
Seed of 50 of the F_3 lines of this cross was available also for testing with form 4. The relation of their reaction to form 4 with their reaction to the collection is shown in table 20. Although several deviations from an exact agreement occur, these differences could have resulted from a failure to recover recessive segregates in some cases owing to the small number of plants per line.

Near Immune × Resistant

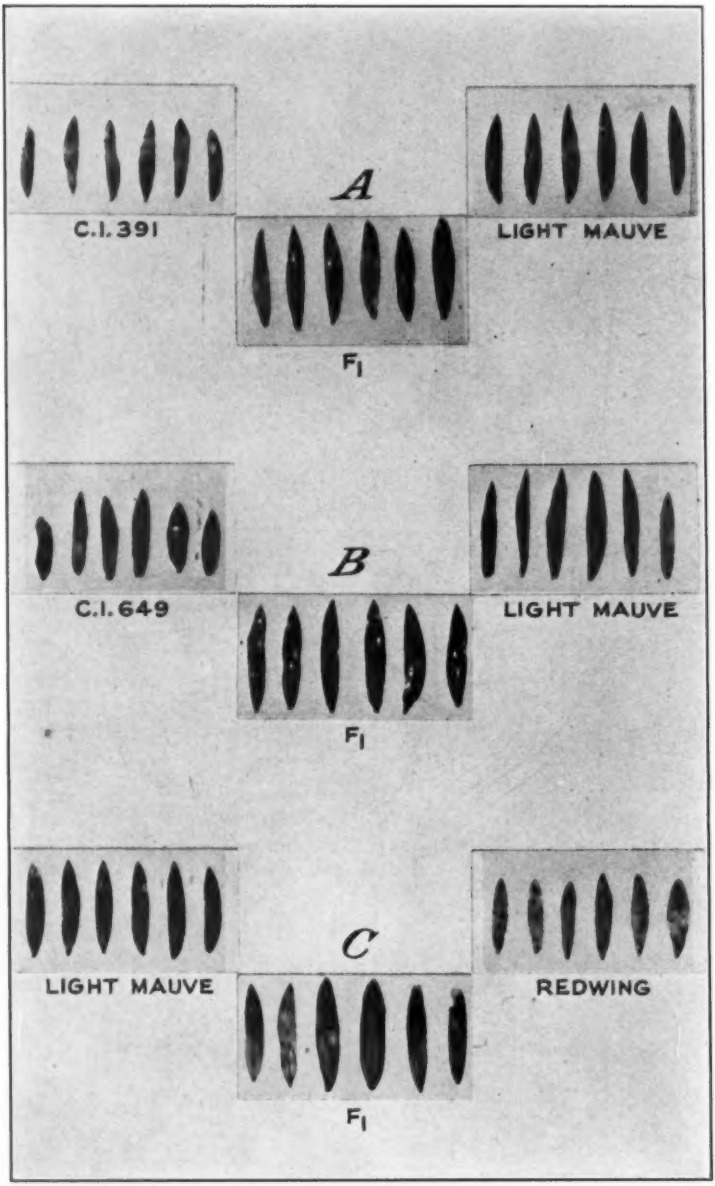
Thirty-four F_3 lines of C. I. 416–3×C. I. 712, averaging 11.6 plants per line, were inoculated with the collection of rust. Because of the overlapping of the near-immune and resistant reaction, it was impossible to distinguish near-immune from resistant segregates. Consequently, the genetic factors differentiating near immunity and resistance could not be determined in this cross. In addition to near-immune and resistant segregates, 3 of the 34 F_3 lines each contained a single semiresistant plant and 1 line contained a susceptible segregate when inoculated with the collection. When plants of the F_3 lines were inoculated with form 4, six lines contained semiresistant and

Table 21.—*Reaction of parents, and of F_1 and F_3 generations of C. I. 712 × (Long × E) to collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—											
	Collection						Form 4					
	0	1	2	3	7	8	0	1	2	3	5	7
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
C. I. 712	14						10	7				
Long × E		12	5				1	12	1			
F_1	4	1					1	1				
C. I. 712	9	12					10	4				
Long × E	5	12	3				14	1				
F_3 families:												
6142–4	3	6	7	1			1	12	3	2		
6142–6	1	2	10	4				3	12	1		
6142–8	1	3	7	3				12	5	1		
6142–9	1	1	4	1				2	6	1	6	2
6142–2	2		8	4			2		8			
6142–5		1	2	2				1	7	3		
6142–10		6	6	3			1	15	3			
6142–7	1	1	9	2		2		1	13	2		
6142–3	1	2	4	4	1			6	5	1	1	
6142–1		2	5	1		1	1	8	3	1		
5139–2	14						10	4	3			
5139–4	18						17					
5139–6	14						12	2				
5139–8	16						14					
5139–10	12						9	2				
5139–15	14						16	1				
5139–17	15						14	3				
5139–3	11	1					10	1				
5139–5	13	2					14					
5139–7	3	3						16				
5139–9	13	3					10	3				
5139–11	15	1					13					
5139–14	12	1					4	2				
5139–16	11	2					1	6				
5139–19	3	2						9				
5139–23	10	1					6	1				
5139–36	6	1					1					
5139–27	12	4					12	2				
5139–28	8	4					8	3				
5139–25	12	1	2				4	2				
5139–24		6	2					4	1			



Reaction to the collection of rust of parents and F_1 plants: *A*, Bolley Golden × Pale Blue; *B*, Bolley Golden × Abyssinian Yellow; *C*, Light Mauve × Bison.



Reaction to the collection of rust of parents and F_1 plants: *A*, C. I. 391 × Light Mauve; *B*, C. I. 649 × Light Mauve; *C*, Light Mauve × Redwing.

two lines susceptible segregates. These semiresistant and susceptible segregates may have resulted from the absence of allelism of factors conditioning near immunity and resistance in the two parents or from the segregation of minor modifying factors. Insufficient data were available in this cross for selecting between these alternatives.

The results obtained in C. I. 712 × (Long × E) are given in table 21.

One striking feature of the reaction of the F_3 lines when inoculated with the collection was observed. In the progeny of F_2 family 6142 the plants were predominantly class 1, 2, and 3 with a few segregates also in class 0. In addition, 3 lines out of the 10 showed susceptible segregates. On the other hand, progeny of F_2 family 5139 were predominantly class 0, 14 of the 21 F_3 families also having segregates in class 1 and 2. This sharp difference between F_2 families indicates that the two F_1 plants were genetically different, probably as a result of heterozygosity for factors for rust reaction of one or both of the parental lines. A similar situation obtained in the material tested with form 4.

Near Immune × Semiresistant

One cross of near immune × semiresistant, i. e., Light Mauve × Redwing, was studied. When inoculated early in the fall, some plants of Redwing were placed in classes 4 and 5 and some were classed as susceptible. However, the cross Light Mauve × Redwing was inoculated on November 23 when the light intensity and duration were much reduced, and at this time Redwing gave uniformly a susceptible reaction, as is seen in table 22.

Table 22.—*Reaction of parents and of F_1 and F_2 generations of Light Mauve × Redwing to a collection of rust in greenhouse*

Parent or generation	Plants showing indicated reaction					
	0	1	2	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Redwing						16
Light Mauve		3	15			
F_1			2	2		
Redwing						18
Light Mauve	17	3				
F_2	113	20	1	4	11	32

In the additional crosses made in 1934, a resistant plant of Light Mauve was used. The four F_1 plants were classed as two class 2 and two class 3 (pl. 3, *C*). Thus, the resistance of Light Mauve was nearly completely dominant to the susceptibility of Redwing.

Since the Light Mauve plants which descended from the original parent of the F_2 and F_3 generations occurred in both class 0 and class 1, it seemed logical in the analysis of the segregating populations to group the class 0 and class 1 segregates. In addition, the results with the F_1 plants indicate that class 3 was not greatly different genetically from class 2 but instead may have represented a fluctuation due to environment. It is interesting to note that the class 1, 2, and 3 plants in the F_2 generation made up 18.1 percent of the near-immune group (classes 0, 1, 2, and 3), whereas 15 percent of the Light Mauve

plants were classified as class 1. Combining the class 5 and susceptible plants, the ratio in F_2 was 138 near immune:43 semiresistant and susceptible plants. χ^2 for fit of this ratio to a 3:1 gave a value of P between 0.50 and 0.70. This indicates that a single major factor pair differentiates Light Mauve and Redwing as regards reaction to the collection of rust. Twenty-six F_3 lines, averaging 7.63 plants per line, were studied and a ratio of 11 near immune:7 segregating:8 semiresistant and susceptible F_3 lines was obtained. χ^2 for fit to a 1:2:1 ratio gave a value of P between 0.02 and 0.05, such a deviation being expected only 2 to 5 times out of 100 trials due to chance alone. However, with the small number of plants per F_3 line, it is probable that some lines were incorrectly classified, particularly those classed as near immune because of failure to recover the expected recessive segregates. Insufficient seed of the F_3 lines of this cross was available for testing with form 4.

Resistant × Semiresistant

The data on rust reaction of the parents, and of the F_1 and F_2 generations of Light Mauve × Bison are presented in table 23.

Table 23.—*Reaction of parents and F_1 and F_2 generations of Light Mauve × Bison to collection of rust in greenhouse and to form 4*

Parent or generation	Plants showing indicated reaction when inoculated with—										
	Collection					Form 4					
	1	2	3	5	7	0	1	2	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Light Mauve	1	6				4	24	6			
Bison			2	8	9				7	26	1
F_1	1	3	1			1	1				
F_2	13	90	22	15	6	9	89	62	6	11	

In the material inoculated with the collection, the five F_1 plants were placed in classes 1, 2, and 3 (pl. 2, *C*), the resistance of Light Mauve being almost completely dominant over the semiresistance of Bison. The overlapping of Bison and the F_1 in class 3 was a source of difficulty in analyzing the data, particularly in the F_2. On the basis of parental reaction, 10.5 percent of the plants of the genotype of Bison should occur in class 3. If the 21 plants in classes 5 and 7 are used as the basis for calculating the F_2 plants with the same genotype as Bison, 2.5 of the class 3 plants should be of that genotype. Combining the remaining class 3 plants with the resistant group, since the data on F_1 indicates that part of the heterozygous plants will also be in class 3, the ratio was 122.5:23.5. χ^2 for fit of this ratio to a 3:1 gave a value of P slightly above 0.01. Twenty-nine F_3 lines were inoculated with the collection and a ratio of 9 resistant : 12 segregating : 8 semiresistant and susceptible lines was obtained. Five of the eight lines classed as semiresistant and susceptible contained class 3 plants, but, since class 3 plants also occur in Bison, this seemed legitimate. When this ratio was compared with a 1:2:1 by means of χ^2, P was between

0.50 and 0.70. The results in F_3 indicate that, although the deviation from the expected was rather large in F_2, Light Mauve is probably differentiated from Bison by a single major factor conditioning resistance to the collection of rust.

Since classes 0, 1, and 2 plants occurred in Light Mauve when tested to form 4, these were combined in analyzing the F_2 data on reaction to form 4. Likewise, classes 3 and 5 both were found in Bison and these two classes were also combined in F_2. This gave a ratio of 160:17. χ^2 for fit of this ratio to 15:1 gave a value of P between 0.05 and 0.10, suggesting that the resistance of Light Mauve to form 4 may be conditioned by duplicate factors. Unfortunately, insufficient seed of the F_3 lines was available for a satisfactory test of their reaction to form 4. Therefore it was impossible to draw definite conclusions regarding the inheritance of resistance to form 4 in this cross.

The reaction of the parents, F_1 and F_2 generations of C.I. 391 × Light Mauve to the collection, is given in table 24.

TABLE 24.—*Reaction of parents and of F_1 and F_2 generations of C. I. 391 × Light Mauve to collection of rust in greenhouse*

Parent or generation	Plants showing indicated reaction				
	1	2	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*
C. I. 391				14	16
Light Mauve	8	26			
F_1		4		1	
F_2	9	67	11	14	10

All plants of Light Mauve were placed in classes 1 and 2, while the plants of C. I. 391 were placed in classes 5 and 7. Of the five F_1 plants, four were placed in class 2 (pl. 3, *A*) and one was placed in class 5. Since C. I. 391 was used as the female parent, it is probable that this single class 5 plant was selfed C. I. 391 rather than a hybrid. In the F_2, combining class 3 with the resistant group and considering classes 5 and 7 together, a ratio of 87 resistant : 24 semiresistant and susceptible plants is obtained. The fit to a 3:1 ratio is good, P lying between 0.30 and 0.50, thus supporting the evidence of a single dominant factor conditioning the resistance of Light Mauve to the collection.

RESISTANT × SUSCEPTIBLE

Two crosses of resistant × susceptible, involving Bolley Golden with Pale Blue and with Abyssinian Yellow were studied. All plants of Bolley Golden inoculated as checks with these crosses were placed in classes 1 and 2, whereas all check plants of Pale Blue and Abyssinian Yellow were in class 7. The hybrid plants, both in F_2 and F_3, occurred in classes 1, 2, 6, and 7. In the analysis of the data, classes 1 and 2 were combined into a resistant group, while classes 6 and 7 were considered as susceptible. This seemed logical not only on the basis of parental reaction, but also because of the sharp difference between the segregates of the two different groups. The data on the reaction of these two crosses to the collection of rust are summarized in table 25.

TABLE 25.—*Reaction of parents and of F_1, F_2, and F_3 generations of Bolley Golden × Pale Blue and Bolley Golden × Abyssinian Yellow to collection of rust in greenhouse*

Parent or generation	Plants or lines showing indicated reaction		
	Resistant	Segregating	Susceptible
Bolley Golden ..... plants	76		
Pale Blue ..... do			70
F_1 ..... do	4		
F_2 ..... do	225		73
F_3 ..... lines	7	16	9
Bolley Golden ..... plants	51		
Abyssinian Yellow ..... do			34
F_1 ..... do	3		
F_2 ..... do	109		36
F_3 ..... lines	12	8	13

In both crosses, the F_1 plants were resistant (pl. 2, *A* and *B*). In the F_2 generation of Bolley Golden × Pale Blue, χ^2 for fit to a 3:1 ratio gave a value of P between 0.80 and 0.90. Comparing the ratio of F_3 lines to 1:2:1 by means of χ^2 gave, in this case, a value of P between 0.80 and 0.90. The total χ^2 for fit to a 3:1 ratio within the segregating F_3 lines was 5.351 and P was in excess of 0.99. Such a good fit is expected less than once in 100 trials. However, the number of plants per F_3 line was only 10.3 and such a low χ^2 value may have resulted, in part at least, from the small numbers.

In the F_2 generation of Bolley Golden × Abyssinian Yellow, χ^2 for fit to a 3:1 ratio gave a P value between 0.95 and 0.98. When the ratio of F_3 lines was compared with a 1:2:1 ratio, P lies between 0.01 and 0.02. However, the average number of plants per F_3 line was only 10.4. Therefore, susceptible segregates would not always be recovered in the progeny of heterozygous F_2 plants, in which case the lines would be classed as resistant. Total χ^2 for fit to a 3:1 ratio within the segregating F_3 lines gave a P value between 0.98 and 0.99. Also in this case the low χ^2 value may have resulted from the small number of plants in the F_3 lines. The preponderance of evidence in these two crosses is in support of the assumption of a single dominant factor conditioning the resistance of Bolley Golden to the collection, a hypothesis suggested by the results obtained in Ottawa 770B × Bolley Golden. Material of these two crosses was likewise tested with form 4, and the data on the reaction of parents and F_1 and F_2 generations are given in table 26.

TABLE 26.—*Reaction of parents, and of F_1 and F_2 generations of Bolley Golden × Pale Blue and Bolley Golden × Abyssinian Yellow to form 4*

Parent or generation	Plants showing indicated reaction			
	0	1	2	7
	Number	*Number*	*Number*	*Number*
Bolley Golden	20			
Pale Blue				18
F_1	4			
Bolley Golden		14	4	
Pale Blue				18
F_2		42	103	39
Bolley Golden		34	3	
Abyssinian Yellow				38
F_2		64	62	53

The 20 plants of Bolley Golden that were progeny of the plant used in the cross with Pale Blue in the summer of 1934 were all class 0 when inoculated with form 4. Likewise, the four F_1 plants were class 0. The 18 plants of Bolley Golden which were progeny of the original parent of the cross from which the F_2 material of Bolley Golden × Pale Blue descended were placed in classes 1 and 2. All plants of Pale Blue were in class 7, i. e., susceptible. Combining the class 1 and 2 segregates in F_2 into a resistant group, the ratio was 145:39. χ^2 for fit of this ratio to 3:1 gave a P value between 0.20 and 0.30. In Bolley Golden × Abyssinian Yellow, combining classes 1 and 2, a ratio of 126:53 was obtained. χ^2 for fit to a 3:1 ratio gave a P value between 0.10 and 0.20.

The association of the reaction of the F_3 lines of Bolley Golden × Pale Blue to the collection with their reaction to form 4 is shown in table 27. It can be seen from these data that the agreement is almost complete. In the one line classed as resistant to the collection and segregating with form 4, only nine plants were tested to the collection. Likewise, in the two lines classed as segregating with the collection and resistant to form 4, only six and four plants, respectively, were tested with form 4. A similar situation is shown in table 27 for the F_3 lines of Bolley Golden × Abyssinian Yellow.

TABLE 27.—*Correlation table showing the reaction of F_3 lines of Bolley Golden × Pale Blue and Bolley Golden × Abyssinian Yellow to collection of rust in greenhouse and to form 4*

BOLLEY GOLDEN × PALE BLUE

Reaction to form 4	Reaction to collection			
	Resistant	Segregating	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Resistant	6	2		8
Segregating	1	10		11
Susceptible			9	9
Total	7	12	9	28

BOLLEY GOLDEN × ABYSSINIAN YELLOW

Resistant	5			5
Segregating	1	6		7
Susceptible			9	9
Total	6	6	9	21

In the one line classified as resistant to the collection and segregating with form 4, only eight plants were tested to the collection. These results indicate that the factor which conditions the resistance of Bolley Golden to the collection also determines its resistance to form 4.

SEMIRESISTANT × SUSCEPTIBLE

The data on reaction of parents and F_1 and F_3 generations of C. I. 479 × Bison and Pale Blue × Bison to the collection, are summarized in table 28.

TABLE 28.—*Reaction* [1] *of parents and of* F_1 *and* F_2 *generations of C. I. 479 × Bison and Pale Blue × Bison to collection of rust in greenhouse*

Parent or generation		Number of plants or lines showing indicated reaction		
		SR	SR, S	S
C. I. 479	plants			33
Bison	do	26		11
F_1	do	4		
F_2	lines	7	20	4
Pale Blue	plants			40
Bison	do			33
F_1	do			5
F_2	lines		27	37

[1] See footnote 1, table 5.

In C. I. 479 × Bison, all of the plants of C. I. 479 were classified as susceptible. The plants of Bison varied from semiresistant to susceptible and the four F_1 plants were placed in class 5, indicating dominance of semiresistance in this case. Of the 31 F_2 lines studied, 7 contained only semiresistant (class 4 and 5) plants, 20 contained both semiresistant and susceptible segregates, and 4 were classed as susceptible. One of the four susceptible lines also contained a single class 5 plant which might have resulted from genetical segregation or which might have been a natural hybrid. In view of the variability of Bison from semiresistant to susceptible, an attempt to give an exact factorial explanation of the results obtained in this cross does not seem expedient. When these F_2 lines were inoculated with form 4, class 3 segregates occurred in 10 of the lines. Likewise, 4 of the 34 plants of Bison were placed in class 3. Again no factorial explanation of the results could be given.

All of the plants of Bison, Pale Blue, and the F_1 of the cross Pale Blue × Bison were classed as susceptible. This behavior of Bison is unique so far as these studies are concerned, although Flor (*3*) reported Bison as susceptible in his studies of greenhouse reaction. The variability of the reaction of Bison from semiresistant to susceptible in other inoculations in this experiment indicates that its reaction may be readily influenced by the environment. Therefore, it is perhaps not surprising to find conditions under which all plants of Bison are susceptible. In the 64 F_2 lines of this cross, 27 contained both class 4 and susceptible plants, the remaining 37 lines being susceptible. Again, in this cross, it does not seem possible to give a factorial explanation of the results.

CROSSES INVOLVING C. I. 649

Since the analysis of the reaction of the check plants of C. I. 649 showed that this variety was quite variable in its rust reaction, it seemed desirable to discuss the crosses in which it was involved together.

The reaction of the parents and F_2 generation of Ottawa 770B × C. I. 649 to a collection of rust is shown in table 29.

The plants of C. I. 649 ranged in reaction from class 1 to class 5. Combining these four classes in the F_2 generation, the F_2 plants are found to occur in the ratio of 426:134:31. χ^2 for fit of this ratio to a

12:3:1 gave a value of P between 0.02 and 0.05. Thus a deviation as great or greater than this would be expected by chance only 2 to 5 times in 100 trials.

TABLE 29.—*Reaction of parents and F_2 generation of Ottawa 770B × C. I. 649 to collection of rust in greenhouse*

Parent or generation	Plants showing indicated reaction					
	0	1	2	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Ottawa 770B	75					
C. I. 649		8	5	35	20	
F_2	426	27	6	51	50	31

In the crosses involving C. I. 649 with Light Mauve and with the three semiresistant varieties, Bison, Redwing, and C. I. 391, considerable killing of the hybrid material as well as the plants of C. I. 649 occurred in the incubators when an incubation period of 48 hours was used. All material of these crosses was replanted and an incubation period of 24 hours was used. Almost no injury occurred with this shorter period in the incubators. However, this necessity of replanting reduced the supply of seed so that insufficient seed was available for testing with form 4. The reaction to the collection of rust of parents, F_1, F_2, and F_3 generations of these crosses is summarized in table 30. No factorial explanation can be given for the results in these crosses, although it seems probable that multiple factors were involved in determining the type of reaction of C. I. 649.

TABLE 30.—*Reaction*[1] *of parents, and of F_1, F_2, and F_3 generations of crosses involving C. I. 649 to collection of rust in greenhouse*

Parent or generation		Number of plants or lines showing indicated reaction						
		R	R,SR,S	R,SR	R,S	SR	SR,S	S
Light Mauve	plants	29						
F_1	do	5						
F_3	lines	17	5	4	1			
C. I. 649	plants	17				2		
Bison	do					25		1
F_1	do	1				4		
F_2	do	50				57		2
F_3	lines	5	9	13	2	1	1	
C. I. 649	plants	9				8		
Redwing	do							22
F_1	do					3		1
F_2	do	17				52		30
F_3	lines		12	11		2	6	
C. I. 391	plants					11		19
C. I. 649	do	34						
F_1	do					4		
F_2	do	43				137		7

[1] See footnote 1, table 5.

CORRELATION OF FIELD AND GREENHOUSE REACTION

For the plant breeder, a knowledge of the extent of correlation of field reaction with the rust reaction of young plants in the greenhouse is of considerable importance. The reaction of the parents

both under field conditions and in the greenhouse has already been discussed. Two varieties, Long × E and C. I. 416–3, which showed a near-immune reaction in the greenhouse, and C. I. 712, resistant under greenhouse conditions, were immune in the field. The reaction of the other varieties in the greenhouse was comparable to their field reaction, except C. I. 649, which was somewhat less resistant in the greenhouse than in the field.

The critical test of whether the same genetic factors govern field and greenhouse reaction is afforded by a study of hybrid material. Such information was available in only two crosses in this study. Progenies of 54 of the 55 F_2 plants of Ottawa 770B × Pale Blue, which were studied in the field, were inoculated in the greenhouse both with the collection and with form 4. Of the 44 F_2 plants that were immune in the field, 11 had only immune progeny in the greenhouse, while the progeny of 33 plants segregated for immune and susceptible. The 10 F_2 plants that were susceptible in the field had only susceptible progeny in the greenhouse. The rust reaction in the field of 99 F_3 lines of Ottawa 770B × Redwing has been discussed. Seed was available for testing in the greenhouse of 92 of these lines and they were inoculated with form 4. The relation between the field reaction and the reaction to form 4 of the F_3 lines is shown in table 31.

TABLE 31.—*Correlation table showing the reaction of F_3 lines of Ottawa 770B × Redwing to rust in the field and to form 4 in the greenhouse*

Reaction to form 4	Lines showing indicated field reaction			
	Immune	Segregating	Rusted	Total
	Number	*Number*	*Number*	*Number*
Immune	27	2		29
Segregating		42		42
Rusted			21	21
Total	27	44	21	92

The results obtained in these two crosses indicate that the same genetic factor conditions the immunity of Ottawa 770B in the greenhouse and in the field.

DISCUSSION

A summary of the reaction to the collection of rust in intercrosses of the immune, near-immune, and resistant varieties and the suggested genotypes of these varieties are presented in table 32.

The results obtained in all of these crosses except those involving Long × E can be explained by assuming factors in two different allelic series, in which *L* and *M* are duplicate factors conditioning immunity from the collection. l^n and m^n are duplicate factors conditioning near immunity, l^n being allelic to *L* and m^n allelic to *M*. l^r and m^r are duplicate factors conditioning resistance to the collection, l^r being allelic to *L* and l^n and m^r being allelic to *M* and m^n. On this basis, susceptible segregates or varieties would carry the recessive allels in each series and would be of the genotype *ll mm*.

TABLE 32.—*Summary of the reaction to the collection of rust in intercrosses of immune, near-immune, and resistant varieties and the suggested genotypes of these varieties*

Variety and genotype	Ottawa 770B *LL mm*	C. I. 438 *LL* $m^r m^r$	Newland *ll MM*	C. I. 416-3 *ll* $m^n m^n$	Long × E $l^v l^v$ $m^n m^n$
C. I. 438 *LL* $m^r m^r$.	Immune				
Newland *ll MM*.	Immune and susceptible.	Immune and resistant.			
C. I. 416-3 *ll* $m^n m^n$.	Immune, near immune, and susceptible.	Immune	Immune and near immune.		
Long × E $l^v l^v$ $m^n m^n$.	Immune and near immune.	Immune, near immune, and a few susceptible.	do	Immune and near immune.	
C. I. 712 $l^r l^r$ *mm*.	Immune, resistant, and a few semiresistant.	Immune and resistant.	Immune, resistant, and susceptible.	Near immune, resistant, and a few susceptible.	Near immune, resistant, and susceptible.
Bolley Golden	Immune, resistant, and susceptible.				
Light Mauve	do				

The proportion of susceptible segregates in the cross of Ottawa 770B × Newland indicated that the immunity of these two varieties was, in each case, conditioned by a single dominant factor, the factors in the two varieties not being allelic. Studies of seven other crosses of Ottawa 770B with resistant, semiresistant, and susceptible varieties confirmed the assumption of a single dominant factor for immunity in Ottawa 770B. Likewise, the results in C. I. 712 × Newland substantiated the assumption of a single dominant factor conditioning the immunity of Newland. On this basis, the genotype of Ottawa 770B could be written *LL mm* and that of Newland *ll MM*. The occurrence of susceptible segregates in C. I. 416–3 × Ottawa 770B indicated that C. I. 416–3 did not carry a factor for near immunity allelic to *L* while the absence of such segregates in C. I. 416–3 × Newland suggested that C. I. 416–3 carried the factor m^n for near immunity which was allelic to the *M* factor carried by Newland.

Susceptible segregates were obtained in C. I. 712 × Newland, suggesting that C. I. 712 did not carry a factor for resistance allelic to the *M* factor of Newland. In Ottawa 770B × C. I. 712, a few semiresistant segregates obtained. These could have resulted from the segregation of minor modifying factors with C. I. 712 carrying the l^r factor for resistance allelic to the factor of Ottawa 770B. On this assumption, susceptible segregates would be expected in C. I. 712 × C. I. 416–3. Actually, a few semiresistant and susceptible segregates were found in this cross, but fewer than would be expected on the hypothesis. It is possible that these segregates were likewise conditioned by modifying factors and that these two varieties carried a factor for resistance not allelic to either *L* or *M*. However, the evidence is not conclusive.

Results in the crosses of C. I. 438 with Ottawa 770B and Newland suggest that C. I. 438 carried the same factor that conditions the immunity of Ottawa 770B and a factor for resistance, m^r, which was allelic to the *M* factor carried by Newland. Thus the genotype of C. I. 438 would be *LL* $m^r m^r$. This was further substantiated by the results in crosses of C. I. 438 with C. I. 712 and C. I. 416–3. No

semiresistant or susceptible segregates obtained in either of these crosses. The behavior in crosses involving Long × E was not entirely consistent. No susceptible segregates were obtained either in crosses of (Long × E) × Newland or Ottawa 770B × (Long × E), suggesting that Long × E carried factors allelic to both the *L* and *M* factors. On this basis, no susceptible segregates would be expected in crosses of Long × E with C. I. 438 or C. I. 712, whereas susceptible segregates were obtained in both crosses. Results in both of these crosses indicated that one or both parents used in making the crosses were heterozygous, a fact which might have accounted for the susceptible segregates.

Crosses of the resistant strain of Light Mauve with two semiresistant varieties, Bison and C. I. 391, indicated that a single dominant major factor determined the resistance of Light Mauve. Likewise, the results in crosses of the resistant strain of Bolley Golden with the two susceptible varieties, Pale Blue and Abyssinian Yellow, were explained by the assumption of a single dominant factor conditioning the resistance of Bolley Golden. In crosses of these two varieties with Ottawa 770B, about one-sixteenth of the F_2 segregates were semiresistant or susceptible, indicating that the factors for resistance carried by these two varieties were not allelic to the *L* factor conditioning immunity in Ottawa 770B. No crosses were available either for determining whether these two resistant varieties, Light Mauve and Bolley Golden, carry the same factor for resistance or for determining the relationship with the *M* factor of these factors for resistance.

The immune strain of Bolley Golden was found to carry a single dominant factor for immunity and another dominant factor conditioning resistance which was hypostatic to the factor for immunity. The results in the cross of the near-immune strain of Light Mauve with Redwing suggested a single factor difference between these two varieties as regards reaction to the collection of rust. No crosses were available for studying the relation of the factors carried by these two strains with the factors for immunity, near immunity, or resistance contributed by the other varieties studied.

A definite factorial explanation could not be given for the inheritance of the C. I. 649 type of reaction. The results suggested that multiple factors might be involved. Likewise it was impossible to place the inheritance of the semiresistant type of reaction on a definite factorial basis.

SUMMARY

The nature and interaction of genes conditioning different types of reaction to rust and the relationship of genes conditioning the same type of rust reaction in different varieties was studied with physiologic form 4 and a collection of rust. Thirty-seven crosses involving 17 strains and varieties of flax were used in these studies.

In the field studies, Ottawa 770B, C. I. 438, Newland, Long × E, C. I. 416–3, C. I. 712, and one strain of Bolley Golden were found to be immune from rust. One strain of Bolley Golden and the varieties Light Mauve and C. I. 649 were resistant, Bison and Redwing were semiresistant, and C. I. 391, Pale Blue, and Abyssinian Yellow were moderately susceptible. The immunity of Ottawa 770B in the field was conditioned by a single dominant factor.

In the greenhouse studies with the collection of rust, Ottawa 770B, Newland, C. I. 438, and one strain of Bolley Golden were immune. Long × E, C. I. 416–3, and one strain of Light Mauve were near immune. C. I. 712 and strains of Bolley Golden and Light Mauve were resistant. Bison, Redwing, and C. I. 391 were semiresistant and Pale Blue and Abyssinian Yellow were susceptible. C. I. 649 gave a mixed reaction varying from resistant to semiresistant.

Immunity was dominant to near immunity, resistance, and susceptibility, and resistance was dominant to semiresistance and susceptibility in the crosses used in this study.

The reaction to the collection of rust of crosses involving Ottawa 770B, Newland, C. I. 438, C. I. 416–3, and C. I. 712 was explained by assuming factors in two different allelic series, *L* and *M*. *L* and *M* are duplicate factors conditioning immunity. l^n and m^n condition near immunity, l^n being allelic to *L* and m^n allelic to *M*. l^r and m^r condition resistance to the collection, l^r being allelic to *L* and l^n and m^r allelic to *M* and m^n. On the basis of this hypothesis, the genotype of Ottawa 770B is *LL mm;* Newland, *ll MM;* C. I. 438, *LL* m^rm^r; C. I. 416–3, *ll* m^nm^n, and C. I. 712, l^rl^r *mm*.

Results in crosses involving Long × E indicated that it probably carried the l^n and m^n factors. The occurrence of susceptible segregates in C. I. 438 × (Long × E) and C. I. 712 × (Long × E) may have resulted from heterozygosity of the parental material.

A single major factor apparently conditioned resistance to the collection both in the resistant strain of Light Mauve and in Bolley Golden. Crosses with Ottawa 770B indicated that neither of these varieties carried a factor for resistance allelic with *L*. No crosses were available for determining the relationship of the factor or factors in these varieties with the *M* series of alleles.

The immune strain of Bolley Golden carried two factors, conditioning immunity and resistance, respectively, to the collection. A single major dominant factor conditioned the near immunity of the strain of Light Mauve to the collection. No data were available for determining the relationship of factors in these two strains with *L* or *M* series of alleles.

No factorial explanation could be given for the inheritance of the C. I. 649 type of reaction or of the semiresistant reaction.

Reaction of the parents to form 4 was similar to their reaction to the collection. Bison and C. I. 649 seemed somewhat more resistant and Redwing and C. I. 391 somewhat more susceptible to form 4. This difference may have been conditioned by environmental factors.

In general the reaction of hybrids to form 4 was similar to their reaction to the collection. Results indicated that the same factors conditioned the immunity of Ottawa 770B and Newland from the collection and from form 4. The factors determining immunity and resistance to the collection in crosses involving the immune strain of Bolley Golden likewise conditioned immunity and resistance, respectively, to form 4. In hybrids involving the resistant strain of Bolley Golden, the same factor conditioned resistance both to the collection and to form 4. There was evidence that the m^r factor of C. I. 438, which conditioned resistance to the collection, determined immunity from form 4. Two duplicate factors appeared to be conditioning the resistance of Light Mauve to form 4. However, this evidence was not conclusive.

The factor conditioning the immunity of Ottawa 770B in the field also determined its immunity in the greenhouse both when inoculated with the collection and with form 4.

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TWIG LESIONS AS A SOURCE OF EARLY SPRING INFECTION BY THE PEAR SCAB ORGANISM[1]

By J. R. KIENHOLZ, *junior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture;* and LEROY CHILDS, *superintendent, Hood River Branch Experiment Station, Oregon State Agricultural College*[2]

INTRODUCTION

Pear scab (*Venturia pyrina* Aderh.) has been known to be present in the Hood River Valley of Oregon for the last 20 years but has become of commercial concern only since 1932. During that year two pear (*Pyrus communis* L.) orchards produced as much as 80 percent of scabby fruit, and in 1934 plantings at a distance of nearly 2 miles from the original infection centers showed some scab. The disease has increased also in parts of southern and western Oregon and western Washington.

In the course of experiments for scab control in the Hood River Valley, conducted by the writers, several new facts concerning the life cycle of the pear scab organism in relation to control measures were established. The early dispersal of conidia from overwintering scab lesions on twigs, the effect of spray materials in relation to this phase of the disease, and the comparative importance of conidia and ascospores in initiating primary infections are especially worthy of consideration in this connection.

TWIG INFECTION

HISTORICAL REVIEW

Frequent reference has been made to shoot infection on trees since Aderhold (*1*)[3] first described pear scab as occurring on young branches. English writers generally agree that conidia produced from this type of carry-over are almost entirely responsible for primary spring infections, and that ascospore discharge from leaf material is of only minor importance (*5*, *14*). The more outstanding contributions are reviewed by Marsh (*14*). In Australasia, workers generally have found that ascospores appear to be more important in initiating primary infections than conidia produced by the fungus overwintering on the young shoot growth. Data substantiating this view are found in publications by Cunningham (*3*), Curtis (*4*), Hearman (*10*), and Pittman (*16*) and in additional references cited in these papers. Dowson (*6*), however, emphasizes the importance of twig lesions as a source of infection at the time when sprayings are practically over.

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[3] Reference is made by number (italic) to Literature Cited, p. 681.

Very few published data have appeared in the United States, especially in recent years, on the twig phase of pear scab, or on entirely effective control measures. Duggar (*7*) has given a general survey of earlier work in this country, and Heald (*9*) has supplemented it. Smith (*17*) found that under California conditions primary infections were due to spores liberated from scab pustules on wood growth of the past season. His observations and experimental work were substantiated by the results of commercial field tests for the control of the disease. Thomas (*18*), however, concluded that ascospores are the principal source of primary infections in California since a search in commercial orchards in 1929 revealed that the great majority of the current season's lesions had been circumscribed by a cork layer before the end of the growing season.

Differences of opinion appear to exist also among investigators of the Pacific Northwest. Fisher and Newcomer (*8, p. 7*) state:

> In the treatment of pear scab it is important to dispose of all possible sources of infection, and to this end twig cankers, if present, should be removed in pruning and the infected wood burned. Some disposition should also be made of fallen leaves which harbor the fungus over winter, and which are the most important source of early spring infection.

Although not ignoring the function of this ascospore material, Jackson (*11*) pointed out that the disease was more difficult to control in Oregon where twig infections were present, and that several seasons might be required to rid the orchard of this source of infection.

Other than a brief statement by Marsh (*14*) on twig scab control, the writers' preliminary report (*13*) appears to constitute the only record of the effect of sprays on this phase of the disease. Pathologists have tended to base control recommendations on results obtained with the closely related apple scab, but such recommendations have sometimes been found of doubtful application to pears, especially in relation to twig lesions as overwintering sources of infection.

VARIETAL SUSCEPTIBILITY

Approximately half the pear acreage of the Hood River Valley is planted to Anjou, a variety very susceptible to twig attack. Easter Beurre (planted mainly as a pollinator), Flemish Beauty, and Forelle are also very susceptible, but they constitute a minor portion of the plantings in this locality.

Fruit of the Bartlett, the second leading variety, is often slightly affected, especially when interplanted among other heavily infected trees, but twig infections are extremely rare. Bosc is intermediate between Anjou and Bartlett in respect to both fruit and twig attack.

The fact that various degrees of susceptibility have been assigned to these same varieties in other regions indicates that environmental responses or specialized strains of the organism may exert an influence on infection.

In a small planting bordering the Hood River Valley, Bartlett twigs have been found severely scabbed. The greater precipitation and humidity in this section appear to be the factors that allow the parasite to attack this variety so severely.

CYCLE OF INFECTION

New growth of susceptible twigs may be infected at any time during the growing season, but infection occurs more commonly in the Hood

River Valley during spring months when rainfall is frequent. During spring and summer months, new lesions appear merely as small blister-like cushions, often with a prominent lenticular spot at their centers, or on certain varieties as shallow spore-producing stromata. Occasionally the host forms a corky layer beneath the cushion or stroma and partially sloughs it off during the current season. This type is

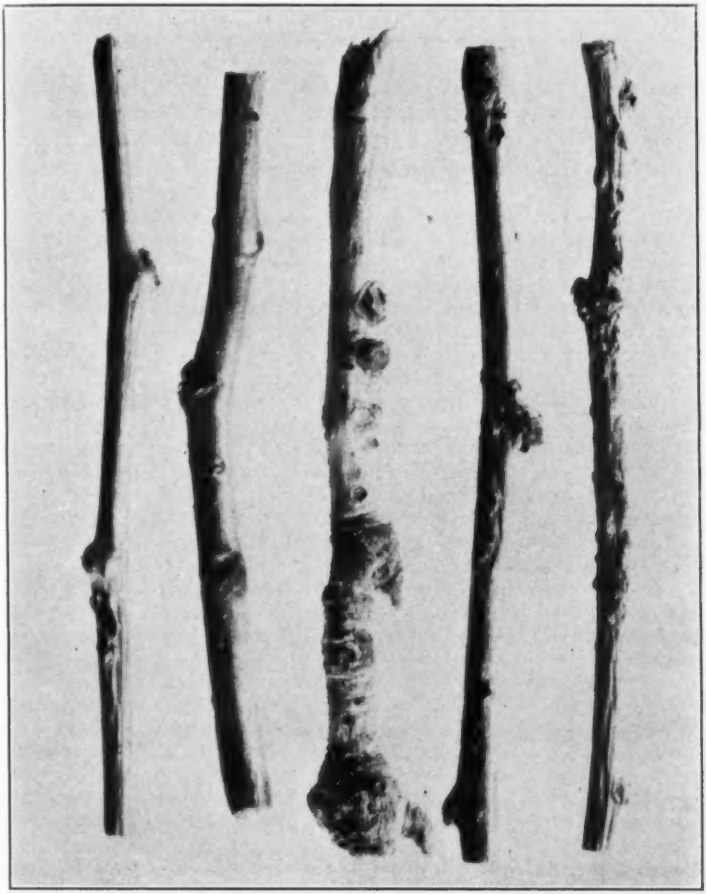

FIGURE 1.—Pear scab on Anjou twigs. Middle twig shows depressions remaining on 2-year-old wood after the scab spots have been sloughed off. From material collected during May.

more easily seen, appearing as a small cankerlike injury. After the primary establishment of the fungus it usually remains more or less inactive until winter, when the trees become dormant. Active enlargement of the fungus fruiting structure then occurs, and by early spring conidial formation has started. Dissemination of these conidia takes place during rainy periods throughout the season or until the

pustules become sloughed off by renewed tree activity. (For a detailed account of the histological features of this cycle, see Marsh's article (*14*), which is substantiated by the writers' observations.) These twig infections (fig. 1) are generally sloughed off during the growing season or before the tree again becomes dormant, although occasionally a few remain partially attached and contain viable conidia the next season. In this case the fungus penetrates the host barrier and may form a new pustule unless a second abscission layer is successful in arresting its growth. Oftentimes 4- and 5-year-old wood still shows evidence of previous infections in the form of circular depressions. Only a short period of activity of these twig pustules is necessary to cause primary spring infections, a fact which Thomas (*18*) appears to have overlooked.

EXPERIMENTAL METHODS

Three orchards in the Hood River Valley were examined in 1934 and two of them again in 1935 to discover at what time primary infection occurred and which spore form was involved. The first orchard was severely infected in both years, and previous studies showed that humidity had reached 100 percent on all but four nights during the preceding summer. Scab had been present for several years, and conditions for its development were ideal, since 100 percent of the unsprayed fruit was affected. In the second orchard, which was believed to represent more closely the general run of orchards in which scab had obtained a foothold, less than 50 percent of the unsprayed fruit had been scabby the previous year. The Oregon State Experiment Station's orchard at Hood River, where scab had never been present, was used as a check plot.

Spore traps were made by tying together, back to back, two slides with outer surfaces coated with petrolatum. Three traps per tree were tied in a vertical free-hanging position at heights of 2, 8, and 16 feet above ground in a representative tree of each orchard. One square inch of each slide was examined under the microscope each week or after periods of heavy rain, for the purpose of recording spore catches.

Ascospore discharge records from overwintered leaves, brought to the laboratory and exposed to natural conditions, were also kept. Five leaves bearing perithecia were placed in a shallow box on a natural orchard soil covering. Ordinary glass slides were then placed directly over each leaf on narrow wood supports to keep them from being in contact with the leaf surface. This procedure is essentially the one described by one of the writers (*2*) in a report of apple scab studies. Discharged spores readily stuck to the glass surface. These slides were examined at the same periods as the spore traps hanging in the orchard. Because of the numbers involved, however, the average number of ascospores caught per square millimeter was used to bring these values in line with other charted data.

SEASONAL DEVELOPMENT AND SPORE LOAD IN RELATION TO SCAB

Records of the weather and of the volume of spores in relation to the prevalence of pear scab in the experiments of 1934 and 1935 are shown graphically in figures 2 and 3. Weather data in the charts are given

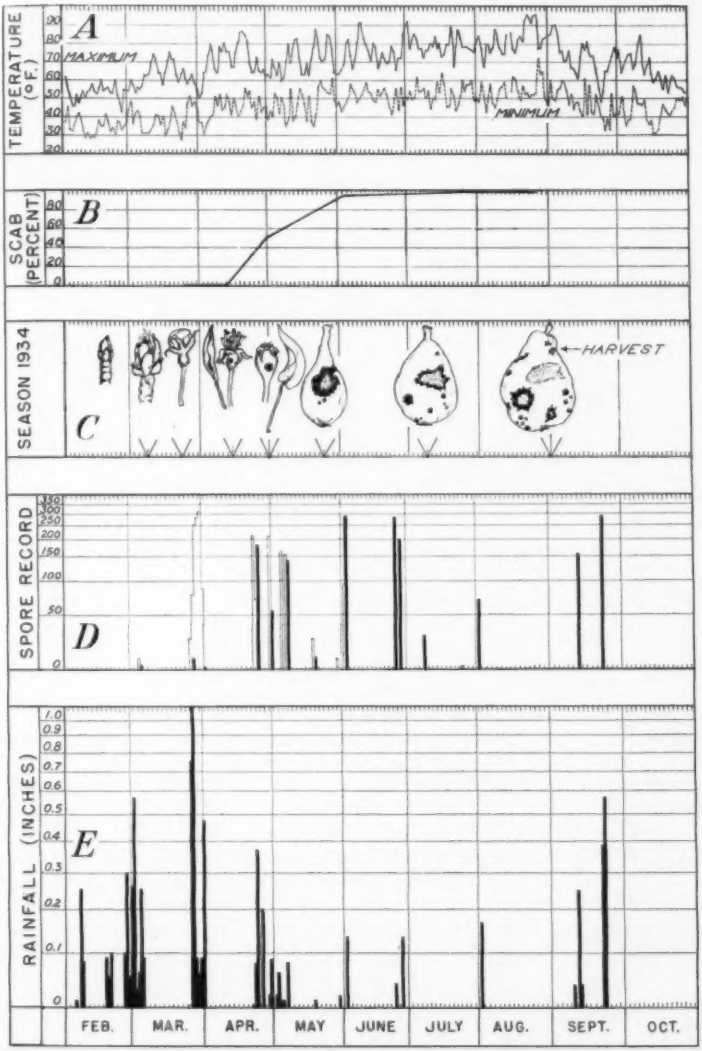

FIGURE 2.—Weather and spore records in relation to pear scab, 1934: *A*, Maximum and minimum temperatures; *B*, scab infection on fruit of unsprayed trees; *C*, stages of development and infection of buds, blossoms, and fruit; *D*, record of spores; light lines coming to a point represent number of ascospores per square millimeter from captive leaves; black bars represent number of conidia per square inch (6¼ square centimeters) caught in orchard; *E*, record of rainfall.

for the station at Hood River, since records are unavailable for the other orchards in which experiments were conducted.

SPORE RECORDS FOR 1934

It has been pointed out by previous workers that ascospore discharge occurs only during rainy periods and that conidia of the fungus are

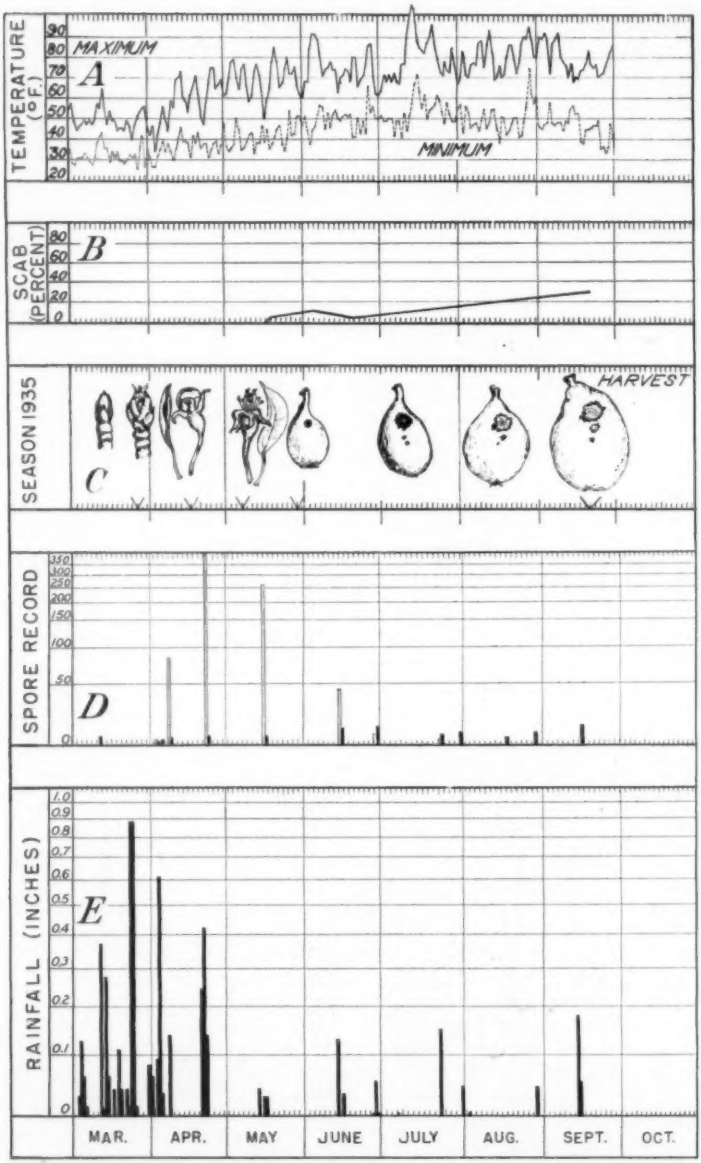

FIGURE 3.—Weather and spore records in relation to pear scab, 1935: *A*, Maximum and minimum temperatures; *B*, scab infection on unsprayed trees; *C*, stages of development and infection of buds, blossoms, and fruit; *D*, record of spores; light lines coming to a point represent number of ascospores per square millimeter from captive leaves; black bars represent number of conidia per square inch (6¼ square centimeters) caught in orchard; *E*, record of rainfall.

readily dislodged by moisture but not by wind alone. Figure 2, *D* and *E*, shows this relation for ascospores from captive leaves and for conidia caught on slides hanging in heavily infected trees during the 1934 season at Hood River. Although ascospores were caught in large numbers on slides placed directly above overwintered leaves, only three were captured during the entire season on the traps hanging in the trees. These three were recorded April 30, June 4, and June 25. Conidial catches in the trees, however, were comparatively large. A local shower fell in the orchard July 9, which explains why conidia were caught at that time. Although total rainfall was slightly higher in this orchard than at the Hood River station orchard, except for July 9, the periods of distribution were the same.

The first catch of conidia was made during the rainy period starting February 26. It is doubtful whether infection could have occurred at that time, since the buds were not open (fig. 2, *C*). Unusually high temperatures during the early spring months, however (fig. 2, *A*), favored rapid growth, so that by March 26, the start of the next rainy period, which yielded the first conidial catch of any consequence, the young leaves and flower buds were exposed.

An examination was made each day to determine the incubation period of the organism. The first scab symptom, a slight greenish fuzz, was found on young fruits April 14, 19 days after the beginning of the second rainy period, which, as previously stated, coincided with the first conidial catch of any consequence. Leaf infections were not found until a few days after the fruit-infection stage and were never numerous except on unsprayed trees. Figure 2, *B*, illustrates the importance of twig lesions as primary infection sources on Anjou pears. It can be seen that even though the spore record shows a comparatively small catch of conidia, half of the fruits on unsprayed trees became infected from these sources.

These primary spots on fruits and other susceptible tissues produced new conidial spore material in such amounts that, with the following rainy period, practically all fruits became infected. Conidial spore catches on the traps in the orchard, of course, increased accordingly. Counts of infected fruits during several stages in their development showed that new spots appeared on susceptible varieties following each rainy period of sufficient duration to allow the fungus to become established. With certain varieties, such as Bosc, the fruits had developed such resistance by the time they were one-third grown that new infections were extremely rare. Scab spots already present gradually died out, and little evidence of scab was seen at harvest unless the fruit had become misshapen. Bartlett pears, which have never become excessively scabby in the Hood River Valley, exhibited a high degree of resistance throughout their cycle of development when associated with severely scabbed Anjous. It appears at this time that, even with an abundant source of spore material, Bosc pears require protection only during early spring, whereas control can be obtained on Anjous only by complete protection throughout the season.

Figure 4 records catches of conidia from the moderately infected orchard. Direct comparisons of unsprayed trees of the heavily and the moderately infected orchards cannot be made, since the owner of the latter did not wish to leave the fruits unprotected. By observing pears in the poorly sprayed treetops, however, it was roughly

estimated that approximately half as much scab developed in this orchard as on the unsprayed fruit of the heavily infected orchard.

No scab spores were caught on slides in the orchard which had not contained scab, and which was some distance from any infected orchard.

It was assumed that if the original spore load from infected wood could be reduced the percentage of scabby fruit showing primary lesions and the chances for reinfections to occur would be lessened accordingly. Figure 5 represents results from two trees that had received no spray in 1933 and that were used to test this assumption. One was left unsprayed; the other received three lime-sulphur sprays in 1934. It can readily be seen that infection was reduced, but reduction of fruit scab approximated only 50 percent because of poor spraying. Figure 4 contrasts the same unsprayed tree with one in a moderately infected orchard receiving three early sprays of a partially effective fungicide. Conidial catches were greatly reduced, but practically half the fruits became scabby because of infections resulting when spray coverages were largely dissipated.

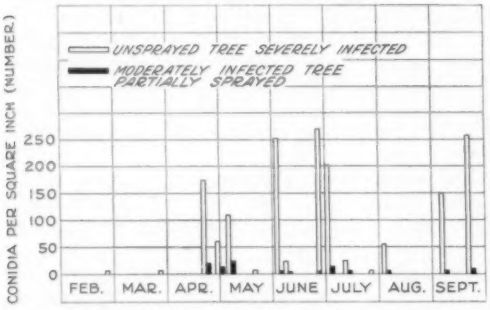

FIGURE 4.—Relation of orchard infection to conidial catch, 1934. (Periodic amounts of rainfall for figs. 4, 5, and 6 may be determined by referring to fig. 2.)

Figure 6 compares conidial numbers caught on slide traps suspended on unsprayed trees at three elevations. The lower and middle heights yielded similar conidial ratios throughout the season, whereas the highest trap was noticeably most free from conidia at each period. Since conidia are washed downward by rains, they naturally are found in greater numbers at the lower tree levels; but it should be borne in mind that a few infected fruits or twigs in the top of the tree are more favorably situated to scatter conidia to healthy fruits than are those situated lower down. Since so few ascospores were caught in the trees, they were considered of minor importance in causing the heavier infection nearer the ground, where, because they are produced in leaves on the ground, they might be of importance if present in larger numbers.

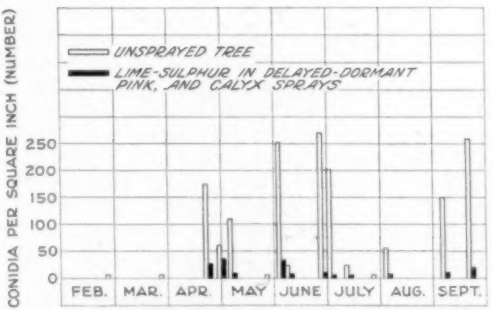

FIGURE 5.—Effect of spray on dispersal of conidia, 1934.

SPORE RECORDS FOR 1935

The year 1935 threatened to be an exceptionally bad scab year, since new wood was heavily infected and the fungus had become established in practically all sections of the valley. Scab, however, did not develop to any considerable extent, for the following reasons:

(1) Before the trees became dormant the previous fall, 80 percent of the twig scab infections became sloughed off or inactivated. (Note the number of inactive scab pustules on twigs of the unsprayed plot in table 2.)

(2) In early spring there was only one rain sufficient to cause scab infection. Where spray recommendations were followed, there was nearly complete protection. (See fig. 3.)

(3) Few new infections appeared following the light rains in May, June, and July, principally because infective material was scarce. Inoculum for subsequent infections was much decreased by the dropping off of infected fruits at the time of the "June drop." Practically all fruits showing pedicel infections were lost at that time.

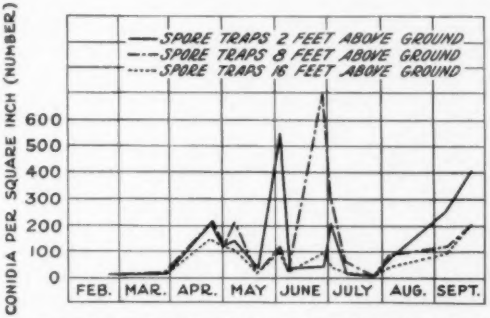

FIGURE 6.—Relation of height of traps to conidial catch, 1934.

Prospects for a severe scab season were somewhat dissipated after the discovery that a large percentage of the twig lesions had become inactivated. For previous experiments to be substantiated it would have to follow that a correspondingly smaller number of conidia would be available for primary infections. That this was actually true can be determined from figure 3, *D*. It should be noted that actual conidial catches early in the 1935 season were slightly less than in the previous year, but the potential sources would have been far greater (see tables 1 and 2) if the wood lesions had overwintered in an active state. An increase in conidial material from secondary infections was small, owing to weather factors previously mentioned. As a result, fruit was much cleaner at harvest than during the previous season when secondary conidial material became increasingly larger up to midseason. Numerous primary infections and favorable moisture conditions later were responsible for the rapid increase of scab in 1934.

Three ascospores constituted the total catch for the 1934 season on nine traps hanging on trees in the orchard. Nine were captured during 1935 in comparable trials from March 1 to the last of May, five of which were recorded on April 21. The primary fruit infections appeared on May 15.

Neither conidia nor ascospores were captured in the check-plot orchard where scab had never been present.

The results of the writers' experience in orchard spraying on a commercial scale during past years correspond very closely with those reported above. When a thorough and properly timed spray was

applied before primary infections had become established and was followed later with a reasonable spray program, satisfactory control of scab was realized at harvest. If, however, the first spray was poorly applied or improperly timed, so that a few primary infections occurred in spite of careful and frequent subsequent sprayings there was considerable scabby fruit at harvest.

CONTROL OF TWIG SCAB

The data given above and observations made during past years indicate that primary spring infections resulted largely from conidia derived from active lesions on scabbed wood of the previous season's growth, and that these conidia were being dispersed even before susceptible tissues were exposed. Ascospore discharge was also recorded from the overwintered leaves, but whether this would occur every year remains to be determined. It is possible, however, that if early sprays are timed by ascospore discharge in orchards where active twig scab occurs, primary infections could already result from twig conidia before the regular spray schedule begins. It has been repeatedly pointed out for both pear and apple scab that the amount of early infection usually influences the number of scabby fruits present at harvest. Any practice that eliminates or checks dispersal of these twig conidia, then, is of paramount importance in control.

EFFECT OF SPRAYS

Data on the effect of fungicides in controlling twig scab are extremely meager. In the orchard used for scab-control experiments, twig infections were exceedingly common and evidence of their existence could be seen on wood several years old when spraying tests were started. Some variation in the total number of shoot pustules per tree occurred, but the orchard could be classified as severely infected and favorable for scab development.

A spray schedule consisting of lime-sulphur 1–12 applied in the delayed-dormant stage and of other materials applied in the pink and calyx stages was employed during the 1933 and 1934 seasons. One additional cover spray was applied in 1933 and two in 1934. Twig counts in the spring following such treatments yielded evidence that sprays measurably control the twig lesions (tables 1 and 2). It should

TABLE 1.—*Effect of sprays on pear twig scab the year following their application, 1933–34*

1933 spray treatment (Delayed-dormant lime-sulphur 1–12 plus 3 later applications)	Scab pustules, 1934			Total twigs infected
	On 50 twigs	Average, per twig	Maximum on any twig	
	Number	*Number*	*Number*	*Percent*
None (west check)	364	7.3	29	90
None (east check)	230	4.6	30	86
Copper oxide-lime-bentonite (2–4–2–50)	30	.6	10	16
Copper phosphate-lime-bentonite (2–4–2–50)	16	.3	3	20
Wettable sulphur no. 1 (10–100) in pink and calyx stage plus bordeaux 3–6–50 in 2 later covers	74	1.5	25	34
Wettable sulphur no. 1 (10–100)	44	.9	7	42
Wettable sulphur no. 2 (10–100)	62	1.2	17	42
Flotation sulphur (6–100)	25	.5	5	28
Lime-sulphur (½–50) and wettable sulphur no. 1 (10–100)	11	.22	4	12
Lime-sulphur (1–50) in pink, wettable sulphur no. 1 (10–100) later	20	.4	12	10
Lime-sulphur (1–50) in pink and calyx, then wettable sulphur no. 1 (10–100)	3	.06	2	4
Lime-sulphur (1–50) in pink, calyx, first cover, then wettable sulphur no. 1 (10–100)	8	.16	2	14

TABLE 2.—*Effect of sprays on pear twig scab the year following their application, 1934–35*

1934 spray treatment (Delayed-dormant lime-sulphur 1-12 plus 4 later applications)	Orchard no.	Scab pustules (1935)						Total twigs infected
		On 50 twigs		Average per twig		Maximum on 1 twig		
		Active [1]	Inactive [2]	Active	Inactive	Active	Inactive	
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Check, no spray	1	41	429	0.82	8.58	7	30	88
	2	122	486	2.44	9.72	9	32	100
Zinc sulphate-lime (4–4–50) plus lime-sulphur (1–100) in covers	1	16	21	.32	.42	3	12	18
	2	33	33	.66	.66	4	4	60
Copper silicate (3–100); thereafter 1½–100	1	1	7	.02	.14	1	2	12
	2	6	6	.12	.12	1	2	22
Bordeaux-lime-bentonite (1–2–1–50)	1	1	0	.02	0	1	0	2
	2	1	3	.02	.06	1	1	8
Copper phosphate-lime-bentonite (2–4–2–50)	1	0	2	0	.04	0	2	2
	2	2	1	.04	.02	1	1	6
Copper oxide-lime-bentonite (2–4–2–50)	1	5	3	.10	.06	1	2	12
	2	14	29	.28	.58	2	4	48
Flotation sulphur-bentonite (3–3–50) (1 pound lime)	1	3	4	.06	.08	1	1	14
	2	1	8	.02	.16	1	2	14
Lime-sulphur (1–100) pink; wettable sulphur no. 1 (10–100) thereafter	1	6	1	.12	.02	1	1	14
	2	5	5	.10	.10	1	1	18
Lime-sulphur lime-bentonite (½–1–2–50)	1	1	1	.02	.02	1	1	4
	2	1	1	.02	.02	1	1	4
Wettable sulphur no. 1 (8–100)	1	6	31	.12	.62	1	10	26
	2	16	44	.32	.88	2	4	62
Bordeaux (1–50) plus oil (½–100) in pink; (1–100) thereafter	1	1	3	.02	.06	1	2	4
	2	1	0	.02	0	1	0	2

[1] Active scab pustules.
[2] Sloughed or inactive pustules.

especially be noted that the wettable sulphur types of materials were much less effective in this control than the more caustic sulphurs or chemicals giving a longer coverage. The same general trend was apparent in fruit scab control. Where mild sprays were used during the early stages of growth it was very noticeable that control of scab on both fruit and twigs was comparatively poor, whereas their substitution at later periods showed less difference.

Figure 5 shows that conidial catches were greatly reduced on sprayed trees. To determine the exact cause of this decrease in conidial numbers early in the season, twigs with active scab pustules were brought into the laboratory and sprayed with certain fungicides. Table 3 gives the results of this test. Lime-sulphur completely inactivated such pustules by an actual "burning out" action. The entire spore-bearing surface was killed and the stroma soon became flat and crusty and did not revive when placed in a moist chamber. In contrast to this, although other fungicides tested caused a considerable decrease in active conidial material where the fungicide was in actual contact with the spore-bearing parts, penetration under the epidermal covering of the pustule was much inferior to that of lime-sulphur. Pustules sprayed with these chemicals, moreover, partially revived in moist chambers and produced some conidia. The same results were observed under field conditions.

TABLE 3.—*Effect of sprays on conidia in pustules on excised twigs*

Spray treatment	Spore germination	Location of spores in pustule	Spray treatment	Spore germination	Location of spores in pustule
Apr. 29, 1935			1934		
	Percent			*Percent*	
Check, no spray	76	Various points.	Wettable sulphur (10–100)	70	Edge.
Lime-sulphur (1–15)	0	Edge.		3.2	Center.
	0	Center.			
Bordeaux mixture (4–4–50)	45	Edge.			
	2.5	Center.			

These results indicate that lime-sulphur applied after twig lesions are open and active should largely eliminate this source of infection. The majority of pustules at Hood River during past years opened before blossom buds became exposed. A small percentage, however, opened during or after this period. It was known from previous experience that lime-sulphur could not be used with safety on Anjou pears in this locality after the bud scales had dropped. For this reason it was believed that best results would be obtained in scab control by delaying the initial lime-sulphur application until the bud scales were just ready to drop. Where it is possible to use this caustic spray on other varieties at a later date, successful control should be possible in one season. Pustules that opened late or those that remained active during the summer contained viable spores at harvest and formed dangerous sources for fall fruit infections. Water sprouts remained susceptible to infection late in the season and required protection where scab was a problem.

A secondary influence of spray materials was apparent on sprayed trees. It appeared that during the spring the host was able to circumscribe scab lesions on twigs more quickly on sprayed trees than on those receiving no treatment. Whether this action was due to a partial killing of the parasite or to a favorable effect on the host was not determined.

RESIDUAL EFFECT OF SPRAYS

The severely infected orchard used for part of these experiments offered an extreme test of the cumulative value of proper and well-timed spray applications. When a part of this orchard was taken over by the writers for spray tests at the end of the 1933 season, 68.3 percent of the commercially but improperly sprayed fruit and 100 percent of the unsprayed pears were scabby. In the 1934 tests the applications of four sprays of various materials, following the delayed-dormant lime-sulphur spray, reduced scab to as low as 12 percent when the stronger fungicides were used. This plot was given back to the grower for the 1935 season because of certain conditions complicating records on fruit russet. However, comparison of this plot with the remainder of the orchard in the less severe scab year of 1935 showed that an average infection of 1.8 percent occurred in the previously well-sprayed section (including all materials tested), while 28 percent of scab was present in the part sprayed entirely by the grower during both years. These results, in conjunction with data given in tables 1 and 2, seem to warrant the conclusion that this

residual effect of spray materials was almost entirely due to the reduction of twig infections, since few ascospores were caught in the trees during either year.

Effect of Dormant Sprays

The possibility of killing the parasite on twigs in its inactive and overwintering form was tried by means of penetrating spray materials developed by Keitt (*12*) for dormant use. During this period the fungus is not exposed, and a penetrating material would be required to reach the vulnerable tissue. Although killing of the fungus occurred, a certain percentage of the pustules continued to become active in the spring, even on trees showing distinct and severe spray injury in the form of partially killed limbs. It is quite possible, however, that if further improved or used in different combinations such materials may find a useful place in the spray schedule for pear scab control. This point needs further investigation.

Effect of Cultural Practices

Certain horticultural practices may influence twig scab infections. The cutting out of infected shoot material has been generally recommended as an aid in controlling pear scab. This practice may be helpful in eliminating some of the original spore load, but even then it is usually not complete enough to be entirely effective without additional protection to new growth. Pruning stimulates the production of new wood, which must be protected to prevent reinfection. Since watersprout types of growth are especially susceptible to shoot infection, however, their removal from the central parts of the tree should be practiced. Pruning so as to produce the most open type of tree without sacrificing bearing surfaces appears to be most desirable from the standpoint of tree vigor, thorough spraying, and partial elimination of spore material.

Unpruned trees and trees very low in vigor have been found to show less infection, since they fail to produce much new and succulent growth.

DISCUSSION

The results obtained on the relation of twig lesions to primary scab infections on pear trees are in accord with work done in England, and indicate that these lesions furnish the bulk of material for early infection under certain environmental conditions. Observations and the spraying of commercial orchards in Oregon during the last 4 years have further substantiated these data. A few twig pustules are easily overlooked in an orchard, but they may be surprisingly persistent in dissemination of the parasite and when favorable environmental conditions are at hand may cause serious epidemics. Where these twig infections are absent it has been relatively easy to control pear scab, even when ascospores from overwintering leaves have been plentiful. The latter, however, should not be ignored, since a certain percentage do find their way to susceptible tissues. Results from widely scattered countries suggest that environmental factors may influence their dissemination. The work of Wiesmann (*19*) and Palmiter (*15*) has shown that specialized strains of pear and apple scab exist, a fact that

gives further importance to this sexual stage in the possible production of new forms.

The long, dry growing season of 1934 was of special interest in its relation to twig attack. Most of the pustules occurred at the bases of the current season's twigs, indicating that infection had taken place soon after they started growth. Twig lesions resulting from infections during the early part of 1934 were sloughed off or became inactive before the trees became dormant, and the majority failed to produce conidia in the spring of 1935. During years with shorter growing seasons and more rainfall, however, the host appeared to be unable to circumscribe incipient infections. As a result, practically all twig lesions remained active and produced conidia the following spring. This may explain the conflicting observations made by Smith (*17*) and Thomas (*18*) in California in different years. It may also explain the occasional and sudden decrease in scab following several seasons in which the disease was difficult to control.

The pronounced effect of spray materials in preventing twig infections and in keeping the old lesions from functioning as infection sources seems conclusive, and spraying appears to offer the most economical and practical means of coping with this phase of the disease. Since the more effective materials can be applied to certain varieties with safety only before the blossom cluster buds become exposed, they should be applied at that time carefully and thoroughly. When properly applied even mild fungicides give considerable protection from reinfection to new twig growth.

SUMMARY

Since 1932 pear scab has become a serious factor in pear production in the Hood River Valley of Oregon. The number of primary infections appeared to correlate closely with the amount of twig infections present. Primary spring infections resulted largely from conidia in overwintering pustules on the previous season's wood rather than from ascospores, and few of the latter were ever trapped in trees. Moreover, conidia were being dispersed before bud tissues were exposed.

Early sprays should be timed by conidial dispersion from twig lesions, where these occur, rather than by ascospore discharge, because infection results from these twig spores before ascospores are matured. Consistent and thorough spraying during the growing season largely prevented twig infections. Early-season sprays were more important in this district for control of twig scab as well as of fruit scab, because more precipitation occurred early in the season and because a certain amount of host resistance became apparent after that time.

Lime-sulphur was effective in "burning out" active twig pustules, but it could not be used on tender-skinned varieties after the young fruit was exposed, without causing injury. This fungicide was found to be dangerous if applied after the bud scales had dropped. Applied in the delayed-dormant stage, lime-sulphur reduced primary spore numbers so that additional sprays gave satisfactory protection against reinfection.

Environmental factors play an important role in natural control.

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PHYSIOLOGIC RACES OF USTILAGO HORDEI[1]

By V. F. TAPKE

Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Covered smut of barley (*Ustilago hordei* (Pers.) Kell. and Sw.) in the United States has long been recognized as an important hazard in the culture of barley (*Hordeum vulgare* L.). In the years from 1918 to 1935 for which records are available,[2] its estimated toll averaged over 2¼ million bushels annually, and twice during these years the estimated annual loss exceeded 5 million bushels. Despite recent notable advances in the control of barley covered smut through seed treatment, as shown by Leukel (*10*),[3] relatively little progress has been made in combating the disease through selecting and breeding barleys having inherent resistance to various physiologic races in the smut species. Until recently, progress in this direction had been almost completely blocked by the lack of an effective and easily applied method of artificial inoculation of the seed for producing high percentages of smutted plants. Despite the general belief of long standing that barley is inoculated, in threshing, by the spread of the spores to the surface of the seed, the artificial blackening of seed with millions of spores usually has failed to result in high percentages of smutted plants. Jensen (*9*) first reported this difficulty nearly 50 years ago. In 1934, the writer (*15*) devised a spore-suspension method of inoculating seed barley in which spores are washed beneath the hulls. The method is similar in principle to Haarring's (*5*) "evacuation" method of inoculating oats with smut, but it employs no vacuum or nutrient solution. The spore-suspension method is effective, fairly easy to apply, and approaches the natural method of inoculation as recently reported (*17*). The way thus was opened for the studies on physiologic races presented herein.

PREVIOUS INVESTIGATIONS

Faris (*3*, *4*) first reported physiologic races in *Ustilago hordei* in 1924. Five pathogenic races were isolated. Rodenhiser (*11*), in 1928, described seven cultural races, and a further test of two of these

[1] Received for publication July 9, 1937: issued December 1937. Investigations conducted in cooperation with the New York (Cornell) and North Carolina Agricultural Experiment Stations. A number of the smut collections and lots of barley seed used in the experiments were collected by the late J. A. Faris, formerly senior pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry. The writer also has had access to the records of Faris' experiments on physiologic races of *Ustilago hordei*, conducted at Kearneysville, W. Va., in 1932 and 1933, in cooperation with the West Virginia Agricultural Experiment Station, and at Fargo, N. Dak., in 1933, in cooperation with the North Dakota Agricultural Experiment Station.

[2] UNITED STATES BUREAU OF PLANT INDUSTRY. ESTIMATE OF CROP LOSSES DUE TO PLANT DISEASES. 1917 U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bull. 2:1–18, 1918. [Mimeographed.]

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[3] Reference is made by number (italic) to Literature Cited, p. 691.

showed differences in their pathogenicity on Lion and Himalaya barleys. Recently Aamodt and Johnston (*2*) found two races in Alberta, Canada. Apparently this report completes the list of available contributions. The fact that there have been relatively few investigations of physiologic races of *U. hordei* doubtless is due largely to the difficulties with seed inoculation, as noted previously.

MATERIALS AND METHODS

The experiments were conducted at Ithaca, N. Y., during the 3-year period 1934–36. Eight pure-line varieties of spring barley were used as differential hosts, namely, Excelsior (C. I.[4] 1248), Gatami (C. I. 575), Hannchen (C. I. 531), Lion (C. I. 923), Nepal (C. I. 595), Odessa (C. I. 934), Pannier (C. I. 1330), and Trebi (C. I. 936). The selection of these varieties was based largely on unpublished studies of the late Dr. J. A. Faris. Faris conducted an experiment at Fargo, N. Dak., in 1933, to determine suitable differential barleys and physiologic races of *Ustilago hordei*. Thirty varieties inoculated with each of 65 smut collections comprised the test. The maximum percentage of smutted heads was 28, in the variety Odessa, which proved to be susceptible to every smut collection. In most of the other varieties the maximum smut infection rarely exceeded 15 percent. Although the data were inadequate to warrant definite conclusions, they were useful to the writer in indicating that certain collections of smut were representative of distinct races and that the varieties named above should make good differential hosts. Faris (*3*, *4*) also had found that Hannchen and Nepal well differentiated two smut races which he numbered "1" and "2."

For differential hosts, the writer chose, so far as possible, from the varieties listed by Harlan and Martini (*6*), those that are outstanding for certain characteristics and representative of some barley-growing region of the world. In the course of study, the following additional species and varieties also were tested for their suitability as differential hosts: Hillsa (C. I. 1604), *Hordeum deficiens* Steud. (C. I. 668–1), *H. intermedium* Koern. and Wern. (C. I. 4377), Lyallpur (C. I. 3403), Club Mariout (C. I. 261), Oderbrucker (C. I. 1529), Summit (C. I. 929), and White Smyrna (C. I. 910). The first four proved too highly resistant to be useful, and the remainder failed to further or better differentiate any of the smut races thus far isolated with the varieties selected. Twenty-eight winter varieties or selections were also tested to determine their possible value in differentiating physiologic races of covered smut.

The 8 selected varieties, during the 3-year test period, were inoculated with each of 200 collections of covered smut obtained from 26 States. Preparatory to inoculation, seed of the differential hosts was soaked in a formaldehyde solution (1 part of formaldehyde to 320 parts of water) for 2 hours, washed in running water for one-half hour to remove all traces of formaldehyde, and spread in thin layers until

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

thoroughly dry. Each year five check rows of uninoculated seed of each variety so treated produced smut-free plants. In view of the occurrence of mixed collections of the different barley smuts, the spores of the collections used each year were first examined to determine the character of the epispore and the type of germination on 2-percent potato-dextrose agar. Later, the smutted heads resulting from seed inoculation with each collection were examined in the different varieties. All collections that were suspected or determined to be other than true barley covered smut were eliminated.

In 1934, 1935, and 1936, respectively, 75, 60, and 65 new smut collections were tested. The 1934 collections comprised six races; in 1935 two other races were isolated, but in 1936 no additional races were obtained. Two collections of each of the six races obtained in 1934 were further tested in 1935 and 1936, and two collections of each of the two races obtained in 1935 were again tested in 1936. In these further tests of the isolated races, the inoculum of each race to be used in the succeeding year's test was collected on the same date and subsequently stored in a cool room. Whenever possible, the inoculum was collected from the variety which most clearly differentiated each race to aid in screening out any other races that might have occurred in the original collection. A month or less before seeding, the seed was inoculated by the spore-suspension method previously described (*15*).

The inoculated seed in each year's test was planted at the rate of 6 g to the 5-foot row. The new collections were tested in duplicated systematically distributed rows. In the further tests, in 1935 and 1936, of previously isolated races, triplicated systematically distributed rows were employed. In preparing the inoculum, in inoculating, and in planting the seed, adequate precautions were observed to prevent mixing of spores of different collections. The smut percentages obtained were based on counts of the total number of heads per row in 1934 and 1935 and of 300 heads per row in 1936.

In the analysis of data three infection classes were used, as follows: 0–5 percent infection=resistant class (R); 6–35 percent infection=intermediate class (I); 36 percent infection and above=susceptible class (S).

The classification is arbitrary and the limits of the infection classes have been selected to fit the present data. The purpose of the classification is to present in a convenient way the host reactions in the present study that differentiated the various races of covered smut. As shown in table 1, increase in susceptibility frequently is accompanied by an increase in the variability of infection. The progressive widening in limits of the intermediate and susceptible classes accommodates these variations. In some previous studies of cereal smuts, narrower infection ranges have been considered significant for separating physiologic races. In the present study, however, the variations in different years and between replications in a single year frequently were too great to warrant the use of a classification with more than three classes of infection. In the three seasons in which the tests

were conducted at Ithaca, N. Y., extremes of drought, precipitation, and heat occurred during the growing seasons of 1934, 1935, and 1936, respectively. Under such conditions, the separation of smut races doubtless should be based only on differences in pathogenicity that are fairly wide and reasonably consistent.

In each of the 3 successive years of the experiment, the maximum percentages of smutted heads in the susceptible variety Odessa were 62, 54, and 50, respectively. In Nepal, the maximum percentages were 60, 45, and 58, respectively. The conditions for infection thus appear to have been adequate for the differentiation of physiologic races.

RESULTS

IDENTIFICATION OF PHYSIOLOGIC RACES

The annual and average percentages of smut produced by each of the eight races of *Ustilago hordei*, in 2 or 3 years of the tests, are given in table 1. In each year the two collections of each race produced similar results. Data for only one collection of each race therefore are recorded. As noted previously, some of the smut collections used were collected by Faris. These collections were numbered, but their physiologic race identity was unknown except that one collection was labeled "form 1" and another "form 2." In the present experiments the reaction of these races on the varieties Hannchen and Nepal was similar to that described by Faris (*4*), and Faris' numbers were therefore retained. The varietal reactions which differentiate the races are given in table 2, and this is followed by a key to facilitate the identification of the races. Table 2 and the key show that the eight smut races may be identified with only five of the eight varieties used. However, two of the varieties omitted, Gatami and Trebi, have given good differential reactions with some races and have been useful in confirming their identity. Odessa, also omitted from the key, is needed to perpetuate race 8.

The total number of collections obtained from each of the 26 States that served as sources of the collections, and also the number of races and their relative prevalence in each of these States as indicated by the collections used in these experiments, are given in table 3.

TABLE 1.—*Percentages of smutted heads in 8 varieties of spring barley inoculated with 8 physiologic races of Ustilago hordei, and grown at Ithaca, N. Y., 1934–36*

Race No.	Year tested	Smutted heads in—							
		Excelsior (C. I. 1248)	Gatami (C. I. 575)	Hannchen (C. I. 531)	Lion (C. I. 923)	Nepal (C. I. 595)	Odessa (C. I. 934)	Pannier (C. I. 1330)	Trebi (C. I. 936)
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	1934	0.0	0.0	8.2	0.0	0.0	34.3	0.0	9.8
	1935	0	0	18.6	0	0	36.9	0	2.4
	1936	0	0	18.8	0	0	44.9	0	2.2
	Average	0	0	15.2	0	0	38.7	0	4.8
2	1934	4.0	12.8	2.2	10.2	40.5	41.2	0	3.6
	1935	0	7.7	0	17.8	43.0	30.8	0	0
	1936	0	13.5	0	12.5	58.2	38.4	0	0
	Average	1.3	11.3	7	13.5	47.2	36.8	0	1.2
3	1934	20.0	0	0	7.1	60.0	25.0	4.2	0
	1935	30.3	.6	0	15.7	45.1	40.8	0	.6
	1936	29.3	0	0	11.6	46.5	49.3	0	0
	Average	26.5	.2	0	11.5	50.5	38.4	1.4	.2
4	1934	0	0	8.8	0	18.8	45.2	13.8	24.2
	1935	.9	0	13.2	0	32.0	29.7	13.5	9.6
	1936	0	0	10.5	0	33.1	29.5	6.9	4.2
	Average	.3	0	10.8	0	28.0	34.8	11.4	12.7
5	1934	0	0	0	15.6	0	61.8	0	27.9
	1935	0	0	0	19.1	0	27.0	0	18.8
	1936	0	0	0	12.7	0	21.4	0	10.4
	Average	0	0	0	15.8	0	36.7	0	19.0
6	1934	0	0	30.4	25.5	0	52.6	0	45.8
	1935	0	0	17.4	20.2	0	34.3	0	29.5
	1936	0	0	32.1	19.3	0	50.2	0	31.1
	Average	0	0	26.6	21.7	0	45.7	0	35.5
7	1935	.8	0	0	0	24.3	53.5	1.5	3.6
	1936	.3	0	0	0	29.5	26.9	0	.3
	Average	.6	0	0	0	26.9	40.2	.8	2.0
8	1935	0	0	0	0	.5	39.7	0	0
	1936	0	0	0	0	0	28.3	0	0
	Average	0	0	0	0	.3	34.0	0	0

TABLE 2.—*Reactions of 5 varieties of spring barley which differentiate 8 physiologic races of Ustilago hordei*

Race No.[1]	Reaction[2]—				
	Excelsior (C. I. 1248)	Hannchen (C. I. 531)	Lion (C. I. 923)	Nepal (C. I. 595)	Pannier (C. I. 1330)
8	R	R	R	R	R
1	R	I	R	R	R
7	R	R	R	I	R
4	R	I	R	I	I
5	R	R	I	R	R
6	R	I	I	R	R
2	R	R	I	S	R
3	I	R	I	S	R

[1] The race numbers are presented in the order given to facilitate comparison with the key to 8 physiologic races of *Ustilago hordei* that follows.

[2] R (resistant) = 0–5 percent of smutted heads; I (intermediate) = 6–35 percent; S (susceptible) = 36 percent or more.

Key to eight physiologic races of Ustilago hordei

	Physiologic race
Lion resistant:	
Nepal resistant:	
Hannchen resistant	8
Hannchen intermediate	1
Nepal intermediate:	
Pannier resistant	7
Pannier intermediate	4
Lion intermediate:	
Nepal resistant:	
Hannchen resistant	5
Hannchen intermediate	6
Nepal susceptible:	
Excelsior resistant	2
Excelsior intermediate	3

TABLE 3.—*Number and distribution of physiologic races of barley covered smut in 200 collections from 26 States*

Location	Collections of race No. —								Total collections
	1	2	3	4	5	6	7	8	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Arizona					1				1
California	1		1		34	1			37
Colorado						1			1
Georgia	3								3
Idaho		1	1		2	13			17
Illinois						1	2		3
Iowa			1			9			10
Kansas			1		1	1			3
Louisiana						1			1
Michigan						1			1
Minnesota	1					19			20
Missouri			1			1			2
Montana				1		2			3
Nebraska						9			9
New York						10			10
North Carolina	2					1			3
North Dakota				1		18			19
Oklahoma						1			1
Oregon		1			1				2
South Dakota						2			2
Texas	1								1
Utah					1	14			15
Virginia	7					1		1	9
Washington				1	17	5			23
West Virginia								1	1
Wisconsin						3			3
Total	15	2	5	3	57	114	2	2	200

The data in table 1 show, in general, a high degree of consistency in the percentages of infection with each of the races during the 2 or 3 years of the test. In some instances certain races produced a low percentage of smutted heads in certain varieties in 1934 but did not cause smut in these varieties in the following years. This may have been due to mixtures in the original collections that were screened out as a result of passage through selected hosts in the following years.

Despite the fact that environmental conditions were marked by unusual variations in temperature and precipitation in the three seasons in which the tests were conducted at Ithaca, the spore-suspension method of seed inoculation proved effective and the degree of smut infection was reasonably uniform.

Two facts are apparent from table 3: (1) The wide distribution of race 6 and (2) the predominance of race 5 in California and Washington.

WINTER VARIETIES AS DIFFERENTIAL HOSTS

In the fall of 1935, the 28 winter varieties or selections listed in table 4 were inoculated with the eight races of covered smut and sown in triplicated, systematically distributed rows at Statesville, N. C. In other respects the general conduct of the test was similar to that with the spring barleys. The results of the test are given in table 4. In general, these winter barleys displayed little clear-cut differential reaction to the smut races except that about two-thirds of them were resistant to race 2. The Nakano Wase selections, including Esaw, however, were moderately susceptible to race 2 and highly resistant or immune to all other races. The Smooth Awn selections 86 and 203 were the most uniformly resistant, showing less than 5 percent of heads smutted by any of the races. Unfortunately, Esaw and the Nakano Wase and Smooth Awn selections are highly susceptible to the brown loose smut (*Ustilago nuda* (Jens.) Kell. and Sw.) which is prevalent in the humid winter barley region.

Doubtless as a preface to further studies on the use of winter barleys as differential hosts, a better knowledge of the influence of winter injury on the incidence of covered smut should be acquired. Tisdale (*18*) and Faris (*3*) have shown that plants of certain winter barleys are more susceptible to winter injury when infected with covered smut than when not infected. In the experiment just described there were wide differences in the degree of winter injury sustained by the different varieties and this may have obliterated differential responses to the races of smut that otherwise would have been apparent.

TABLE 4.—*Percentages of smutted heads in 28 varieties of winter barley inoculated with 8 physiologic races of Ustilago hordei and grown at Statesville, N. C., 1935–36*

Race No.	Smutted heads in—													
	Abiska (C. I. 4106)	Beardless No. 6 (C. I. 2746)	Cussdo (C. I. 895)	Esaw (C. I. 4090)	Gaddis (C. I. 6063)	Garren	Han River (C. I. 2163)	Kashmir	Kentucky No. 2 (C. I. 6148)	Kentucky Smooth Awn (C. I. 6021)	Khayyam (C. I. 1117)	Nakano Wase selection 33	Nakano Wase selection 68	Orel (C. I. 351)
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	10.2	3.3	11.0	0.0	5.3	6.0	22.3	15.1	4.9	36.1	7.9	1.2	0.0	6.8
2	6.3	6.3	1.7	12.3	7.4	1.3	3.1	3.4	.2	9.2	2.4	7.3	10.9	2.1
3	14.4	16.2	6.0	.0	12.2	10.5	16.9	12.3	2.9	34.8	8.1	.0	.0	4.6
4	25.8	17.1	11.5	.0	15.8	13.3	21.2	18.6	7.7	24.6	11.4	.0	.0	16.8
5	40.8	2.6	19.4	.4	7.3	9.4	45.8	30.9	13.8	34.3	25.4	3.0	.6	24.7
6	40.9	5.8	22.3	2.2	5.1	6.5	47.3	28.2	12.8	28.8	26.8	.0	.0	20.3
7	30.5	14.0	28.1	.8	22.5	9.3	38.8	22.4	15.1	27.9	35.0	.0	1.2	22.6
8	39.5	4.9	26.1	1.9	9.2	6.8	32.2	33.1	12.7	28.6	26.6	.0	.0	19.3

TABLE 4.—*Percentages of smutted heads in 28 varieties of winter barley inoculated with 8 physiologic races of Ustilago hordei and grown at Statesville, N. C., 1935–36*—Continued

Race No.	Smutted heads in—													
	Pidor (C. I. 901)	Scottish Pearl (C. I. 277)	Smooth Awn selection 86	Smooth Awn selection 233	Smooth Awn selection 205	Squarehead (C. I. 252)	Tenkaw (C. I. 646)	Tennessee Winter (C. I. 3534)	Tennessee Winter (C. I. 3546)	Tennessee Winter (C. I. 6034)	Tennessee × Abyssinia (37) (C. I. 6296)	Texas Winter (C. I. 554)	Wisconsin Winter (C. I. 2159)	Woods Hooded (C. I. 6235)
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	11.8	12.5	0.0	0.0	0.5	11.5	8.8	16.9	25.1	10.5	22.9	13.6	13.3	13.2
2	11.3	4.0	1.1	1.6	15.0	.9	2.2	2.9	6.8	.5	10.3	2.8	2.9	3.3
3	3.6	7.9	.0	.6	.0	6.8	11.8	12.8	23.7	6.0	16.7	10.1	12.1	11.1
4	19.1	19.4	1.4	.0	.0	21.7	16.6	20.0	37.4	11.9	20.8	16.0	16.6	19.2
5	27.6	25.5	4.7	3.1	5.1	24.7	28.1	25.3	45.7	7.9	29.6	26.1	39.3	10.6
6	39.6	35.0	1.7	.0	.0	36.2	35.8	31.6	57.2	8.5	37.4	38.1	33.3	14.9
7	13.6	31.1	.0	.0	4.8	32.8	37.3	33.2	45.8	10.2	29.4	32.1	34.2	21.1
8	16.5	37.7	.0	.0	2.5	30.7	29.3	27.6	54.5	7.7	35.5	29.1	30.1	11.7

Another factor that seems due to assume greater importance in future studies of physiologic races is the influence of environmental conditions on the response of the host after infection. The results of Faris (*3*) seem to indicate that after the smut has penetrated the seedling environmental conditions may influence the host response in a way other than through winter injury; and, as Faris notes, "the reaction of barley varieties to such environmental changes may not be the same." Aamodt (*1*), Smith (*13*), Rodenhiser and Holton (*12*), and Holton and Heald (*8*) have reported that environmental conditions after infection may affect the incidence of bunt in wheat.

DISCUSSION

Eight physiologic races of *Ustilago hordei* have been isolated on the basis of differences in their pathogenicity on eight varieties of barley. Although sufficient data are not yet available to permit a detailed discussion of the distribution of these races, it seems evident that the race designated No. 6 is the most widely distributed. It was found in 21 of the 26 States from which collections have been obtained. It was also the most frequently collected, occurring in 114 of a total of 200 collections. However, in California and Washington race 5 was outstanding, occurring 34 times in 37 collections from California and 17 times in 23 collections from Washington. The predominance of race 5 in California may be linked with the antiquity and survival of Coast barley in that State. As noted by Harlan and Martini (*7*), when North America was discovered there were no barleys here. The early Spanish missionaries introduced Coast barley into California about 1770, and it is still widely grown there. Of the 37 covered smut collections obtained from California, 26 came from Coast and the two Coast-type varieties Atlas and California Tennessee Winter. Twenty-three of these twenty-six collections proved to be race 5. The early importations of Coast seed in California may have harbored this particular race of smut, which has survived and spread with its susceptible and popular host.

Despite the apparent restriction of certain smut races to limited areas, the probability that the different races will be spread through wind dissemination of spores and through the interchange of infected seed makes it highly desirable to breed barleys resistant or immune to all the known races of smut. To date Pannier (C. I. 1330) has proved highly resistant or immune to seven races and only moderately susceptible to one. *Hordeum deficiens* (C. I. 668–1) and *H. intermedium* (C. I. 4377), used only in the test of 1934, were highly resistant or immune to the six races occurring in the collections of that year. These two and Pannier also have proved highly resistant or immune to two races of the black loose smut (*Ustilago nigra Tapke*) of barley recently described (*14, 16*). In these experiments no smut has been observed in Hillsa (C. I. 1604) and Lyallpur (C. I. 3403), yet the former was inoculated with 65 collections of *U. hordei* in 1933 by Faris and both were inoculated with eight races of *U. hordei* and two of *U. nigra* in 1935 by the writer (*16*). In the light of the results of the present investigation, it appears that physiologic races of *U. hordei* are not more numerous than those of other small-grain smuts and that the breeding of barleys for resistance to covered smut should not be hampered by lack of resistant parental material.

SUMMARY

Eight physiologic races of *Ustilago hordei* were found in 200 collections from 26 States. Race separation was based on differences in pathogenicity on five varieties of spring barley.

The most widely distributed race was collected in 21 of the 26 States. It was also the most generally prevalent race, occurring 114 times in the 200 collections.

In California and Washington another race was conspicuously prevalent and widespread. It occurred 51 times in 60 collections from these States.

Under the conditions of a 1-year test with 28 winter varieties or selections, little clear-cut differential host response to the 8 races of covered smut was obtained. Marked differences in varietal response to winter injury occurred. A better knowledge of the influence of this factor on the incidence of covered smut in winter barleys is needed.

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IMPROVEMENTS IN DETERMINATION OF OIL DEPOSIT ON SPRAYED FOLIAGE [1]

By L. H. DAWSEY, *associate chemist*, and J. HILEY, formerly *assistant scientific aide, Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

A number of methods for the determination of oil deposit on sprayed foliage have been proposed during the last 7 years, but nearly all the reliable ones have been applicable only in the determination of the highly refined petroleum oils. Success with such oils has depended upon the fact that they remain comparatively inert, whereas a large proportion of the natural substances extracted from leaves is destroyed by treatment with strong mineral acid (*4*, *5*, *7*).[2] Under such conditions it is possible to collect and measure the oil in Babcock bottles, and with a knowledge of the surface area of the extracted foliage, to calculate the deposit in terms of the quantity of oil retained per unit of leaf area.

In the case of the less refined petroleum oils (*3*), however, the authors found it necessary to carry check samples, to which known quantities of particular oils had been added, along with the unknown samples, for the purpose of correcting the results both for the plant substances undestroyed by the acid and for certain unsaturates eliminated from the petroleum oil itself by the acid treatment. These operations were no more time consuming than the average chemical determination. In a subsequent attempt to develop an even simpler method, applicable also in the analysis of fatty oils, Dawsey, Cressman, and Hiley (*4*) tried to standardize emulsions upon the basis of the quantities of oil retained by wax-coated plates, but found that the deposit on plate surfaces was not always proportional to that retained by a leaf surface. It was concluded that accurate results were best achieved by oil extraction directly from the foliage, and since then work has been carried forward on such a basis.

The purpose of the investigations described in this paper was to develop improved methods for the determination of oil deposit on foliage which would be applicable to oils of animal and vegetable origin as well as to the nonvolatile petroleum oils. With this objective experiments were carried out to ascertain (1) which was the best solvent to employ in recovering oil from sprayed foliage, (2) the optimum conditions for recovery of 100 percent of the oil, or the total deposit, and (3) the accuracy obtainable under the optimum conditions for oil recovery.

EXPERIMENTAL PROCEDURE

All the tests were performed with leaves from plants of the Old Rose variety of chrysanthemum (*Chrysanthemum hortorum*) which

[1] Received for publication June 22, 1937; issued December 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 701.

had been grown in the greenhouse in pots and allowed to reach a height of about 12 inches. Applications to sprayed foliage were made with emulsions stabilized with bone glue in the proportion of 6 g of glue to 100 cc of oil. The precision sprayer and the spraying methods employed have been described previously (*4*).

EXTRACTION EFFICIENCY OF DIFFERENT SOLVENTS ON NATURAL LEAF SUBSTANCES

The first tests were carried out to determine which of the more common solvents was best adapted for extracting oil from chrysanthemum foliage. It was apparent that the most suitable solvent would be the one in which the spray oil was readily soluble and yet which would dissolve the least material from the leaves.

Table 1 gives a comparison of the quantities of materials extracted by different solvents in the treatment of both powdered leaves and fresh leaf disks in a conventional type of apparatus. To prepare the powdered samples, unsprayed leaves were dried in an electric oven at 110° C., powdered in a mortar, and 0.5000-g samples were weighed out and placed in the thimbles of an A.S.T.M. (American Society for Testing Materials) rubber-extraction apparatus. Extraction was allowed to proceed until the liquids in the siphon cups were colorless. The extracts were filtered into weighed flasks, the solvents evaporated off, and the residues dried to constant weight at 110°. For the leaf-disk samples, 200 fresh disks of 1 cm diameter were cut per sample from the unsprayed plants. It was found that this number of disks, when dried and powdered, weighed 1.068 g. The fresh disks were extracted in a Bailey-Walker extractor for 3 hours, the solvents evaporated in the original weighed extraction flasks, the last traces of volatile substances taken off in a vacuum desiccator at a pressure of 2 to 3 mm of mercury, and the residues determined at constant weight.

TABLE 1.—*Relative quantities of plant substances extracted from dry, powdered leaves and from fresh, green chrysanthemum-leaf disks by different solvents*

Solvent	Powdered leaves		Fresh leaf disks	
	Color of extract	Residue	Color of extract	Residue
		Mg		*Mg*
Petroleum ether	Yellow	44.9	Light yellow	17.1
Carbon disulphide	Dark green	76.9	Brown	34.7
Carbon tetrachloride	do	80.3	Yellow	48.6
Ethyl ether	do	83.5	Green	49.1
Benzene	do	83.9	Yellow	38.2
Methylene chloride	do	90.1	do	
s-Dichloroethylene	do	112.8	do	37.5

Although the powdered leaves were extracted in the A.S.T.M. apparatus and the fresh leaf disks in the Bailey-Walker apparatus, the two sets of results are at least indicative of the relative quantities of leaf substances extractable, since the residues are calculated upon the basis of 1.068 g of dry leaf material continuously extracted for approximately the same period of time.

It is evident that by drying and grinding the leaves before extraction considerably more nonvolatile material is extracted than when green disks are used. In both types of treatment minimum quantities

were taken out by petroleum ether; so this solvent appeared to be the most promising one. In the extraction of the disks the petroleum ether showed only a light-yellow color, indicating that very little chlorophyll had been extracted. This is in keeping with the fact that chlorophyll is contained within the leaf cells, and if the cell walls are not broken practically none is extracted by this solvent. Such small quantities of substances as were obtained with petroleum ether from the fresh disks must, therefore, have consisted of natural waxes and oils from the surface of the leaves. It was concluded from this experiment that petroleum ether would be the most suitable solvent to use in extracting the oil deposit from sprayed foliage.

Rohrbaugh (*7*) claimed petroleum ether to be a superior solvent in the extraction of petroleum oils from dry, powdered citrus foliage.

METHOD OF EXTRACTION AS AFFECTING OIL RECOVERY

English (*6*) stated that oil was completely recoverable from Satsuma orange foliage in a limited number of washings on leaf disks, because subsequent extraction in a Soxhlet apparatus failed to show additional quantities of oil. Later Dawsey (*3*, *5*) made improvements in the English method, based upon the successive washing of disks with ethyl ether, as used in the determination of oil deposit on the foliage of camphor-tree, Satsuma orange, pecan, and chrysanthemum. Rohrbaugh (*7*), on the contrary, claimed that simply washing unground citrus leaves in such a solvent was insufficient for complete recovery.

It was therefore considered desirable to study some of the different methods of extraction and thereby determine the optimum conditions for complete recovery of the deposit from sprayed foliage. To this end experiments were performed to determine the efficiency of oil recovery in washing leaf disks a limited number of times, and also in continuous extraction as is best carried out in a standard type of extraction apparatus.

WASHING METHOD

The first of these tests was a simple washing experiment with ethyl ether and carbon tetrachloride as solvents. Samples containing 200 disks of 1 cm diameter were taken from unsprayed plants and from plants sprayed with a 2-percent emulsion of a highly refined petroleum oil. They were extracted in 125-cc flasks by washing four times with 35 cc of solvent per washing, after which the residues in the total extracts were determined. All residues were determined on a volume basis in Babcock bottles except the initial extractions with carbon tetrachloride, which were determined by weighing. The specific gravity of the oil was 0.8512 as used in converting weight to volume. The oil from the samples of sprayed foliage was calculated by subtracting therefrom the mean of the residues extracted from the unsprayed foliage. Afterward the exhausted leaf disks were dried and reduced to powder, and the washing treatment was repeated to recover any remaining oil. Table 2 shows in the case of both solvents the quantities of residue initially extracted and those extracted in the second treatment.

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TABLE 2.—*Extraction efficiency in washing the oil deposit from mixed leaf disks with ethyl ether and carbon tetrachloride*

ETHYL ETHER EXTRACTIONS

Sample no.	Residue from initial washings				Residue from final washings			
	Sprayed disks	Unsprayed disks	Total oil recovered		Sprayed powder	Unsprayed powder	Total oil recovered	
	*Mm*³	*Mm*³	*Mm*³	*Percent*	*Mm*³	*Mm*³	*Mm*³	*Percent*
1	58.7	4.3			9.2	4.2		
2	59.6	4.3			9.1	3.9		
3	60.8	4.6			8.3	3.7		
4	61.2	4.5			9.3	4.3		
Mean	60.1	4.4	55.7	91.8	9.0	4.0	5.0	8.2

CARBON TETRACHLORIDE EXTRACTIONS

	Mg	*Mg*	*Mg*					
1	47.9	7.0			9.6	2.4		
2	51.9	7.1			11.4	3.1		
3	51.1	7.0			10.7	3.3		
4	53.3	6.6			12.5	2.7		
Mean	51.1	6.9	44.2	86.4	11.1	2.9	8.2	13.6

The total deposit as obtained with ethyl ether agrees closely with that obtained with carbon tetrachloride, the two figures being 60.7 and 60.1 mm³, respectively, when calculated from the data in the table. In the four initial washings ethyl ether was slightly more efficient, but since neither solvent recovered the oil completely, it was concluded that more drastic extraction measures were necessary.

As in this example with chrysanthemum leaves, it is improbable that extractions carried out by Dawsey (*3*), in earlier work on camphor-tree and chrysanthemum foliage, recovered all the deposit. Washing disk samples four times with ethyl ether, however, is seen to give a good approximation of the total deposit on the foliage, and it is unlikely that the general conclusions regarding the insecticidal action of oil sprays drawn in previous work (*1*, *2*) are seriously in error.

CONTINUOUS EXTRACTION

A continuous method of extraction, with petroleum ether as the solvent, was tried next. Samples of green leaf disks taken from plants sprayed with a 2-percent oil emulsion, and from unsprayed plants, were prepared as before and immediately extracted with petroleum ether in the A. S. T. M. rubber-extraction apparatus. After continuous refluxing for 2 hours, the solvent was evaporated down on the water bath, the extracts were transferred to weighed flasks, and the residues were heated to constant weight at 110° C. The residues from the initial extracts were weighed. They were then transferred from the weighing flasks to Babcock bottles and the oil in each sample was redetermined according to the previously developed volumetric method (*3*). Thus, a direct check was obtained upon the accuracy of the weighing method. Both the Babcock measurements and the results obtained in weighing are included in table 3. After the initial extractions, the exhausted leaf disks were dried, reduced to powder, and reextracted to determine whether all traces of the spray oil had been taken out during the first operation. The residues from the

second extractions were estimated by the Babcock method, and these figures are also shown in table 3.

TABLE 3.—*Oil-recovery efficiency of petroleum ether in continuous extraction on fresh leaf disks in A. S. T. M. extraction apparatus*

Sample no.	Residue from initial extraction								Residue from final extraction, Babcock determination			
	Weight determination				Babcock determination							
	Sprayed disks	Unsprayed disks	Oil recovered		Sprayed disks	Unsprayed disks	Oil recovered		Sprayed powder	Unsprayed powder	Oil recovered	
	Mg	*Mg*	*Mg*	*Pct.*	*Mm³*	*Mm³*	*Mm³*	*Pct.*	*Mm³*	*Mm³*	*Mm³*	*Pct.*
1	29.7				32.4				2.5	1.6		
2	39.3	4.6			43.5	3.4			1.7	1.9		
3	38.2	4.5			43.4	3.5			1.8	2.2		
4	38.2	3.9			42.8	3.2			1.7	2.1		
Mean	36.4	4.3	32.1	100.0	40.5	3.4	37.1	98.4	1.9	2.0	0	0

This experiment showed that it was possible to recover 100 percent of the oil deposit, provided fresh leaf disks were continuously extracted in a standard type of apparatus for 2 hours with petroleum ether. This was proved by the fact that further drying, grinding, and extracting of the previously exhausted disks did not give any more oil than was obtained from the unsprayed blanks that were run simultaneously. There was considerable variation from sample to sample, but the quantities of oil as checked by the Babcock method indicated that the weight determinations were reliable. The results of the Babcock determination were slightly lower than those obtained by the weighing method, but this was to be expected since small quantities of oil may have been lost in transferring the residues from the weighing flasks to the Babcock bottles.

TABLE 4.—*Effect of lapse of time between spray application and extraction upon the efficiency of oil recovery from plant foliage*

24 HOURS AFTER SPRAY APPLICATION

Sample no.	Residue from initial extraction								Residue from final extraction, Babcock determination			
	Weight determination				Babcock determination							
	Sprayed disks	Unsprayed disks	Oil recovered		Sprayed disks	Unsprayed disks	Oil recovered		Sprayed powder	Unsprayed powder	Oil recovered	
	Mg	*Mg*	*Mg*	*Pct.*	*Mm³*	*Mm³*	*Mm³*	*Pct.*	*Mm³*	*Mm³*	*Mm³*	*Pct.*
1	62.4	7.6	54.4		66.8	4.2	63.0		1.6	1.7		
2	61.8	8.1	53.8		66.7	3.5	62.9		1.1	1.0		
3	64.2	8.0	56.2		69.8	3.6	66.0			1.4		
4	59.4	8.2	51.4		64.2	4.0	60.4		1.4	.9		
Mean	62.0	8.0	54.0	100.0	66.9	3.8	63.1	99.5	1.4	1.3	0.1	0

6 DAYS AFTER SPRAY APPLICATION

1	55.2	8.3	47.0		56.9	3.7	53.3		1.3	1.3		
2	53.1	8.2	44.9		56.7	4.1	53.1		1.3	1.3		
3	49.9	8.0	41.7		54.0	3.4	50.4		1.2	1.3		
4	56.0	8.3	47.8		59.2	3.2	55.6		1.5	1.1		
Mean	53.6	8.2	45.4	84.1	56.7	3.6	53.1	83.8	1.3	1.3	0	0

Further experiments were conducted to ascertain whether the total deposit could be recovered by continuous extraction with petroleum ether when the extraction was delayed and larger deposits were used (table 4). In these tests a set of plants was divided, some of the plants being sprayed with a 5.0-percent petroleum oil emulsion while others were left unsprayed. The foliage from half the sprayed plants was analyzed about 24 hours after application and the other half were set aside in the greenhouse for analysis 6 days later. In both cases extractions were performed on samples containing 100 disks clipped not more than 3 to 4 hours before extraction. Although only 84.1 percent of the original oil deposit present could be found on the plants 6 days after spraying, reextraction of the exhausted samples, after drying and grinding, showed the oil recovery to be 100 percent. It is thought that the reduction in deposit between the first and the sixth day must have been due to volatilization together with weathering (*8*) of the oil on the plant.

EFFECT OF TREATMENT OF LEAVES BEFORE EXTRACTION

In further experiments on processing leaves before extraction, it was found that only 83 to 88 percent of the total deposit could be recovered during a 2-hour continuous extraction with petroleum ether in the A.S.T.M. apparatus when the disks were dried but not ground to powder. It appears, therefore, that mere drying of the leaves makes the oil even more difficult to extract, so that reduction of the material to a powder then becomes imperative, whereas if the leaves are extracted when fresh, continuous washing is sufficient for complete extraction of the oil deposit. Elimination of the necessity for drying and grinding thus simplifies the work involved in routine analysis.

CHECK ANALYSES

One experiment was carried out with refined corn oil, a typical vegetable oil, to ascertain approximately the errors likely to occur in handling samples during analysis.

Eight samples, each containing 200 disks from unsprayed foliage, were prepared. To four of the samples known weights of corn oil were added; the remaining four samples were used as blanks. The extractions were made under the previously determined optimum conditions, followed by determination of the weights of residues and calculation of the quantities of oil known to be present. Table 5 shows the differences between the calculated quantities of oil present and those known to have been added. The differences calculated on the basis of the amounts of oil present are not more than ± 2 percent. The largest errors occurred in the first two samples where the quantities of oil present were small, but if differences between calculated and known quantities of oil are considered in terms of weight, then it is seen that they fall within the variations occurring among blank samples; hence, the main source of error must be ascribed to variations in the quantities of natural leaf substances in the oil-containing samples rather than to losses of oil in handling.

TABLE 5.—*Calculated quantities of corn oil recovered from samples by petroleum ether extraction as compared with known quantities of the oil added to the samples*

Sample no.	Oil added	Total residue extracted	Leaf substances present [1]	Calculated oil present	Error
	Mg	*Mg*	*Mg*	*Mg*	*Percent*
1	24.9	36.4	11.5	25.3	+1.6
2	56.5	66.8	10.3	55.7	−1.4
3	113.4	124.9	11.5	113.8	+.4
4	182.3	193.5	11.2	182.4	.0
Mean			11.1		

[1] In the blank samples the quantities of leaf substances were 11.0, 10.6, 12.0, and 11.4 mg, with a mean of 11.3 mg.

It is at once apparent that the accuracy in the petroleum ether extraction method for determination of oil deposit depends primarily upon having the same quantities of leaf substances in both blank and oil-containing samples, so that when the blank weights are subtracted from the weights of the oil samples a true figure is obtained for the oil content. This is best accomplished by running blanks simultaneously with oil samples, since blanks are known to vary, depending upon the length of time they are extracted. Moreover, the error in calculating the oil deposit may be larger for small deposits than for large deposits. For example, the following residues were extracted with petroleum ether in a test on unsprayed chrysanthemum foliage where the samples consisted of 200 disks, the time of extraction was 2 hours, and the Bailey-Walker apparatus was used:

Residues, mg	18.4	18.9	20.6	17.6	16.8	18.9	17.5	M=18.4
Difference from mean, mg	0	+.5	+2.2	−.8	−1.6	+.5	−.9	

The extreme difference from the mean was +2.2 in the third sample. If this sample had 30 mg of oil in it, the error introduced in analysis would have been about 7 percent; but if this sample contained as much as 300 mg of oil, the error would have amounted to only 0.7 percent. In practice the petroleum ether method has been found to show extreme errors within this range when a number of samples of sprayed, mixed disks are analyzed together with blank samples. Ordinarily with an average-sized deposit of foliage, however, the error in analysis is small, and may be neglected in view of the fact that very large differences in oil deposit, due to the coverage factor in spraying, completely obscure small errors such as are detectable in the foregoing type of check analysis.

DISCUSSION

Although most of the experimental data were obtained with a refined petroleum oil of 94-percent unsulphonatable residue, the petroleum ether extraction method for determination of oil deposit on foliage, when carried out under appropriate conditions, is applicable to nearly all kinds of nonvolatile insecticidal oils, including the vegetable, animal, and less refined petroleum oils, the last named of which are appreciably attacked by acid treatment in the hitherto employed Babcock methods of analysis. A limitation exists, however, in the determination of semivolatile oils such as pine oil, orange oil,

or kerosene, inasmuch as evaporation losses take place during analysis. The analytical procedure can be followed with standard pieces of laboratory equipment, since it embraces only four operations: (1) Cutting leaf disks in preparation of samples, (2) extracting samples with petroleum ether, (3) evaporating the solvent, and (4) drying residues to constant weight. The extraction can be made practically automatic with the aid of an electrical time switch, so that the labor is expended chiefly in cutting the leaf disks and weighing the residues.

Although the method has been developed primarily for determination of oil deposit on chrysanthemum, it is possible that the same principles can be applied to other kinds of foliage. For example, apple leaves are known to give very much smaller blank residues than chrysanthemum leaves, on the basis of equivalent foliage areas; so the method may be applicable in the analysis of apple foliage with even greater accuracy. Whether the method can be used on citrus is not known, but it is thought that waxy leaves will give larger blanks, thereby lessening the accuracy somewhat.

SUMMARY

An improved weighing method is described for determination of oil deposit on chrysanthemum foliage after spraying with emulsions. The method is applicable to nearly all kinds of nonvolatile insecticidal oils, including both petroleum oils and fatty oils. The procedure consists of four steps: (1) Cutting leaf disks in preparation of samples, (2) extracting samples, (3) evaporating the solvent, and (4) drying residues to constant weight. Recovery of oil from chrysanthemum foliage is 100 percent.

Petroleum ether was found to be the most suitable solvent to use in extraction, because it dissolved smaller quantities of the natural plant substances present on the foliage than other solvents tested.

All the oil could not be extracted by simply washing disks a limited number of times, but when samples contained freshly cut disks and extraction was carried out in a standard type of apparatus for 2 hours, recovery was complete. Drying and grinding of the foliage before extraction was not necessary for complete recovery of deposit even 6 days after spray application.

The accuracy attainable by the method is influenced by the variation in quantities of natural leaf substances extracted from the same number of leaf disks, from sample to sample, and depends upon the size of the oil deposit being measured as compared with the quantity of leaf substances present. The error in calculating low deposits is larger than the error in calculating high deposits. With average-sized and higher oil deposits the error in analysis is small and may be neglected, since very large differences, due to the coverage factor in spraying, obscure any errors detectable by the method of analysis when used in actual practice.

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INTERRELATIONSHIPS OF EGG PRODUCTION FACTORS AS DETERMINED FOR WHITE LEGHORN PULLETS [1]

By I. MICHAEL LERNER, *junior poultry husbandman* and LEWIS W. TAYLOR, *head, division of poultry husbandry, California Agricultural Experiment Station*

INTRODUCTION

It has long been recognized that the egg-producing ability of the domestic fowl depends on a number of inherited and noninherited factors. Since the early attempts to identify and describe these factors a voluminous literature has accumulated. Jull (*7*) [2] has compiled a bibliography which includes the larger proportion of papers published in this field. It must be pointed out, however, that only a few such papers deal with the actual establishment of criteria for these factors. Most of the papers present statistical analyses of observables, described by arbitrarily selected criteria.

Of the attempts to justify the use of one or another measurement on other than empirical grounds, a notable contribution is that of Knox, Jull, and Quinn (*8*), who studied the interrelationships between a number of different heritable and nonheritable factors as well as their relation to the annual egg record. Their conclusions were somewhat at variance with some of the work reported earlier, such as that of Hays and Sanborn (*6*), and with some of the work published since (Lerner and Taylor (*9*, *10*, *11*)). The latter have suggested the use of a number of criteria for the measurement of certain of the factors affecting egg production. The present paper deals with some extensions and further ramifications of these.

Essentially, there are two types of genetic factors that enter into the expression of the egg-laying ability of the fowl. The first type affects the period of time during which the bird is in lay, and includes maturity, persistency, and pauses due to broodiness or other causes. The second type determines the intensity or rate of production of the birds when in lay.

Maturity has been found to be adequately measured by the age at first egg (Warren (*14*); Knox, Jull, and Quinn (*8*); Hays (*5*)). Persistency has been defined differently by different workers, and the grounds on which age or date at last egg are suggested as criteria for its measurement have been discussed by Lerner and Taylor (*10*). Pauses as yet have been defined only arbitrarily, various measurements of pause having been used by Hays (*3*, *4*) and others. The measurement of rate has also been a controversial issue, particularly in the relation of rate to pauses. The use of net rate of production (number of eggs divided by number of days in the laying period considered, less the number of days broody and in pause) has been discussed by Lerner and Taylor (*9*). The application of these criteria and the effect of the observables they measure on egg production form the subject for the analysis presented in this paper.

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[2] Reference is made by number (italic) to Literature Cited, p. 712.

MATERIALS AND METHODS

The trap-nest records of the Single-Comb White Leghorn flock of the Poultry Division were used for the raw data of this analysis. The first-year laying records of two generations of birds were examined and only those of individuals that answered the following requirements were selected: (1) Birds that had completed their full first biological laying year. This eliminated all birds that died or were otherwise disposed of before undergoing a complete molt at the end of the first laying year. (2) Birds that did not pause; that is, had no periods of 7 consecutive days of nonproduction during this time. This requirement was set up to eliminate the question of pause from consideration as far as possible until such time as more precise criteria of pause than those now used become available. It is recognized that the establishment of such a requirement has a certain element of arbitrariness in it, but it is likely that only a few, if any, birds which are genetically pausers would be included in the selected population when 7 days is used as the standard for pause. A possible shortcoming of this method lies in the exclusion of some nonpausing birds and the consequent reduction in the number of birds represented in the populations studied.

These requirements provided a highly selected population of fairly superior layers. Since the hatching season for the 2 years studied was confined to March and April, and the management was uniform throughout, environmental variation may be considered as having been greatly restricted. The analyses for the 67 birds of the 1933 series were made separately from the analyses for the 100 birds of the 1934 series, so that duplicate figures for the 2 years are presented.

The statistical methods used are those described by Ezekiel (*2*), except for the calculation of the coefficient of multiple correlation, which was made in accordance with the method of Wallace and Snedecor (*13*), and the analysis of variance, which was performed in accordance with Snedecor's manual (*12*). A fuller discussion of the various measures of part and partial correlation used will be made in conjunction with the data presented.

FACTORS CONSIDERED

Two measures of annual egg production were used: (1) The production during the 365 days immediately following the first egg, which is the measure most commonly used in breeding and in experimental practice; and (2) the production during the whole of the first biological laying year, from the first egg at sexual maturity to the last egg laid at the onset of fall molt. The latter measure reflects more accurately the inherent ability of the bird to lay, since it lacks the arbitrary element introduced in the first measure.

Since the population selected was nonpausing and nonbroody, the only time factors involved are maturity and persistency. Age at first egg was adopted as the measure for the former and age at last egg for the latter. Material presented elsewhere (*11*) indicates that for the population studied the two measures are equivalent so far as the proportion of variance that is genetic in nature is concerned. Although on the basis of susceptibility to environmental differences, date of last egg was found to be a superior measure of persistency, age at last egg

was used here by analogy with the measurement used for sexual maturity.

The type of measurement for rate of production presented the most difficulty. Though on the basis of considerations previously outlined (*9*) net rate was to be used, the essential point of importance was the selection of the period of time to be used for the determination of rate. As a first step, the calendar year was divided into three 4-month periods, designated winter (November–February), spring (March–June), and summer and fall (July–October). For the sake of brevity the last period will henceforth be referred to as summer.

The rate of production for each bird for each of these periods was calculated and then used individually in combination with the two measures of the time factor. Rate was also measured by single monthly periods but these calculations are not presented, since the establishment of broader principles rather than details is desired here.

As the first step in the analysis proposed, the heritability of the factors to be studied was determined. The simplest way of approaching this problem is through the determination of the relative variance in the observables studied which can be assigned to the differences between the sires of the pullets used in the study. While such variance would represent only a portion of the total genetic variance, a relative estimate of the sires' contribution which finds its expression in the phenotype of the daughters is made. Since the number of different sires involved in the 1933 series was somewhat out of proportion to the number of daughters, the analysis of variance was carried out only for the 1934 series. The results of this analysis are presented in table 1. The lower limit of significance for the degrees of freedom involved is an F value of between 1.65 and 1.88, and it may readily be seen that with two exceptions all the values are highly significant. Winter rate is found to have border-line significance, but undoubtedly passes the test of heritability since only a portion of the genetic variance is

TABLE 1.—*Analysis of variance of the dependent and independent variables with respect to sires, 1934 series* [1]

Source of variance	Degrees of freedom	365-day production		Biological laying-year production		Age at first egg	
		Total squares	Mean square	Total squares	Mean square	Total squares	Mean square
Total	99	101, 093		183, 903		86, 275	
Between means of sires	19	45, 786	2, 409. 8	84, 834	4, 464. 9	22, 584	1, 188. 6
Within means of sires	80	55, 307	691. 3	99, 069	1, 238. 4	63, 691	796. 1
F			3. 49		3. 61		1. 49

Source of variance	Age at last egg		Winter rate		Spring rate		Summer rate	
	Total squares	Mean square	Total squares	Mean square	Total squares	Mean square	Total squares	Mean square
Total	183, 155		5, 511		5, 211		6, 178	
Between means of sires	76, 039	4, 002. 1	1, 698	89. 4	2, 348	123. 6	2, 367	124. 6
Within means of sires	107, 116	1, 338. 9	3, 813	47. 7	2, 863	35. 8	3, 811	47. 6
F		2. 99		1. 88		3. 45		2. 62

[1] F at 5-percent point —1.88; F at 1-percent point —2.42.

represented here. The lack of significance in age at first egg is, however, surprising, not only because there is no question as to its genetic nature (*5, 14*), but also because at least one of the pairs of genes concerned in its determination is known to be sex-linked. Thus the greater portion of the genetic variance exhibited in the daughters should be ascribable to the influence of the sire. A more complete test made on a larger population where pausing pullets were also included in the analysis indicated that a genetic basis for age at first egg cannot be questioned (*11*).

CORRELATION ANALYSIS

The coefficients of simple correlation between the various observables studied are presented in table 2. Instead of the standard errors of these coefficients, the values of $\bar{r}$, representing values most probably true for the universe from which the sample was drawn and calculated in accordance with the formula presented by Ezekiel (*2*), are given. These values serve as an adjustment for the size of the sample used, at the same time indicating the true magnitude of various correlation coefficients with a greater degree of accuracy.

TABLE 2.—*Zero order coefficients of correlation, 1933 and 1934 series*

Variables correlated	1933 series		1934 series	
	r	$\bar{r}$	r	$\bar{r}$
365-day production, age at first egg	−0.348	−0.329	−0.387	−0.376
365-day production, age at last egg	.431	.417	.542	.536
365-day production, winter rate	.495	.484	.543	.537
365-day production, spring rate	.705	.699	.561	.555
365-day production, summer rate	.565	.556	.463	.454
Biological laying-year production, age at first egg	−.430	−.416	−.381	−.369
Biological laying-year production, age at last egg	.592	.583	.691	.687
Biological laying-year production, winter rate	.466	.453	.502	.495
Biological laying-year production, spring rate	.594	.586	.359	.346
Biological laying-year production, summer rate	.484	.472	.380	.367
Age at first egg, age at last egg	.131	.045	.151	.114
Age at first egg, winter rate	.087	0	.138	.095
Age at first egg, spring rate	.020	0	.133	.089
Age at first egg, summer rate	.142	.071	.029	0
Age at last egg, winter rate	.137	.062	.177	.145
Age at last egg, spring rate	.159	.100	.271	.252
Age at last egg, summer rate	.243	.212	.090	0
Winter rate, spring rate	.589	.581	.471	.463
Winter rate, summer rate	.348	.329	.212	.187
Spring rate, summer rate	.745	.740	.905	.904

It is evident from table 1 that the five independent factors exercise an influence on the annual record, measured either by the 365-day or by the biological laying-year production. The time factors are relatively independent of each other, the coefficients of correlation between age at first egg and age at last egg being 0.045 and 0.114 for the two respective series. Age at first egg also shows very low correlation with the three measures of rate, the coefficients varying in magnitude from zero to 0.095. Age at last egg, on the other hand, shows somewhat higher coefficients of correlation with the rate measurements. However, the two series do not give exactly the same results. In order of magnitude, age at last egg is most closely correlated with summer rate

and least with winter rate for the 1933 series, while for the 1934 series it shows a zero coefficient with summer rate and the highest value of 0.252 with spring rate.

Correlation coefficients between rates during the different seasons also show some variation. The highest correlation obtained is between spring and summer rate, followed by that between winter and spring rate, the lowest coefficient being that between winter and summer rate. The latter is, of course, to be expected, since the two periods do not follow one another on the calendar scale, as is the case with the other correlations.

Table 3 presents coefficients of multiple correlation with two and three independent factors at a time and the two production measures in turn as the dependent variable. The most probable values of the coefficients for the universe are once more presented instead of the standard errors, and these values are used for calculations leading to the results presented in the subsequent tables.

TABLE 3.—*Coefficients of multiple correlation, 1933 and 1934 series*

Independent variables	1933 series				1934 series			
	365-day production		Biological laying-year production		365-day production		Biological laying-year production	
	R	$\overline{R}$	R	$\overline{R}$	R	$\overline{R}$	R	$\overline{R}$
Age at first egg, age at last egg	0.594	0.577	0.782	0.775	0.721	0.714	0.848	0.844
Age at first egg, winter rate	.632	.616	.663	.650	.716	.709	.678	.670
Age at first egg, spring rate	.793	.785	.740	.731	.739	.723	.562	.550
Age at first egg, summer rate	.711	.701	.699	.688	.612	.602	.545	.531
Age at last egg, winter rate	.616	.600	.708	.697	.708	.701	.791	.786
Age at last egg, spring rate	.775	.767	.779	.771	.693	.685	.713	.706
Age at last egg, summer rate	.641	.627	.688	.676	.683	.675	.760	.754
Age at first egg, age at last egg, winter rate	.757	.744	.891	.885	.883	.879	.958	.956
Age at first egg, age at last egg, spring rate	.876	.869	.932	.929	.864	.860	.878	.874
Age at first egg, age at last egg, summer rate	.791	.780	.885	.879	.836	.830	.909	.906

It may be noted that the addition of any factor one at a time raises the values of the coefficients of correlation above those obtained with two variables. Using three independent factors at a time further increases the magnitude of the coefficients of correlation obtained. The squares of these coefficients are used as the coefficients of total determination (table 4). They indicate the percentage of variance in the annual record which is accounted for by the variance in the three independent factors.

No uniformity can be observed as to the magnitude of the total determination with respect to the measures used for rate. Thus in the 1933 series the highest coefficients obtained are those for which spring rate is used, accounting for 75.6 percent of the variance of the 365-day record and 86.3 percent of that of the biological laying-year production. In the 1934 series, however, it is the winter rate which gives the highest determination, accounting for 77.3 and 91.4 percent of the variance for the two respective measures of an annual production. On the other hand, the lowest coefficients are obtained when winter rate is used with respect to the 365-day production and summer

rate with respect to biological laying-year production in the 1933 series. In the 1934 series summer rate in the first case and spring in the second yielded the lowest values.

TABLE 4.—*Coefficients of total determination, 1933 and 1934 series*

Dependent variable	Independent variables	1933 series		1934 series	
		Coefficient of total determination	Standard error of estimate	Coefficient of total determination	Standard error of estimate
365-day production	Age at first egg, age at last egg, winter rate.	0.553	19.8	0.773	15.2
	Age at first egg, age at last egg, spring rate.	.726	14.6	.739	16.3
	Age at first egg, age at last egg, summer rate.	.609	18.4	.689	17.8
Biological laying-year production	Age at first egg, age at last egg, winter rate.	.783	16.9	.914	12.9
	Age at first egg, age at last egg, spring rate.	.863	13.5	.764	21.4
	Age at first egg, age at last egg, summer rate.	.773	17.3	.821	18.6

The coefficients of total determination obtained by Knox, Jull, and Quinn (*8*) fall within the range of values presented here. These workers, however, used six independent factors, and the highest coefficient they obtained with a nonselected population of White Leghorns was 0.766. Thus for the above coefficient, date of hatch, date of first egg, age at first egg, length of winter pause, and number of eggs laid in August and September were used. Date of hatch is not a heritable factor; age at first egg and date of first egg are duplicating measures of maturity since the coefficient of simple correlation reported by Knox, Jull, and Quinn between these two variables is 0.901. The number of eggs laid to March 1, though designed to measure intensity, is also a measure of pause, showing a correlation coefficient with length of winter pause of -0.824. Similarly, production in August and September falls short of being an adequate measure of persistency, since it is probable that the element of rate influences it, although no correlation coefficients to test this point are presented by Knox, Jull, and Quinn.

When these workers used only three independent measurements, purporting to measure the same observables as used in this paper, a coefficient of multiple determination of 0.745 was obtained for a White Leghorn flock and of 0.785 for a Rhode Island Red flock, values which are of the same general magnitude as the ones reported here. It should be noted that these independent observables measuring rate (production to Mar. 1) and persistency (August and September production) both form a part of the annual record, thus tending to make the coefficients reported spurious to a degree. In the case of rate as measured here this may also be partially true, since in a nonpausing population net spring rate differs from production for the spring period by a constant (the reciprocal of the number of days in the period). So far as winter and summer rates are concerned, the number of days in the production period is not the same for all birds and hence this criticism is less applicable.

Table 4 also presents the standard errors of estimate for the coefficients of total determination. These have been obtained directly from the latter coefficients in accordance with formula 49 of Ezekiel (*2*). Their magnitude varies from 12.9 to 21.4 eggs for the different combinations presented and their values indicate the degree of reliability to be placed on the multiple correlation values.

Table 5 presents the constants of the multiple regression equations from which estimation of either the 365-day or the biological laying-year production may be made. These were used for the calculation of the part determinations.

TABLE 5.—*Constants of the multiple regression equations, 1933 and 1934 series*

Series	Dependent variable	Age at first egg	Age at last egg	Winter rate	Spring rate	Summer rate	Free term
1933	365-day production	−0.52	0.34	2.25			−3.40
		−.48	.31		2.67		−39.22
		−.53	.31			1.92	45.23
	Biological laying-year production	−.74	.60	2.33			−102.15
		−.70	.57		2.66		−131.94
		−.75	.57			1.90	−46.06
1934	365-day production	−.42	.40	1.55			1.27
		−.55	.36		2.47		−41.61
		−.55	.44			1.68	−8.56
	Biological laying-year	−.64	.69	1.77			−123.08
		−.78	.66		2.40		−149.21
		−.76	.74			1.77	−127.79

RELATIVE IMPORTANCE OF THE INDEPENDENT VARIABLES

The determination of the relative influence of the independent variables on the dependent factor is a somewhat complex procedure. A number of methods are available for this purpose, but it may often happen that different interpretations are possible when different methods are used. When equal or proportional numbers of observations for various subclasses are available, an analysis of variance probably serves the purpose best. However, when such is not the case and when the making of certain assumptions necessary for such an analysis is not a justifiable procedure, other methods have to be used. Ezekiel (*2*) presents four such methods, of which three have been used for this study. The fourth, involving the calculation of coefficients of separate determination, does not give the net action of each of the independents on the dependent variable, and includes the interactions between the independents in the final values obtained. Furthermore, Ezekiel does not recommend the use of this method on a number of other grounds.

The three methods used here involve the determination of the coefficients of partial correlation, the coefficients of part correlation, and of the partial beta coefficients. The relative magnitude assigned to each of the independent variables affords a measurement of their importance in influencing the dependent factor.

The first of these coefficients is a measure long familiar to the workers in the field of inheritance of egg production. The squared coefficient of partial correlation measures the reduction due to the added factor in the variance remaining after the effects of the other independent factors have been accounted for. It is calculated in accordance with Ezekiel's formula 50, and the values obtained appear in table 6 under the heading "Reduction in unexplained variance."

TABLE 6.—*Relative importance of factors affecting the annual record, 1933 and 1934 series*

Dependent factor	Factors already considered	Independent factor	1933 series			1934 series		
			Reduction in unexplained variance	Coefficient of part determination	Partial beta squared	Reduction in unexplained variance	Coefficient of part determination	Partial beta squared
365-day production	Age at last egg; winter rate	Age at first egg	0.303	0.116	0.231	0.554	0.159	0.151
	Age at first egg; winter rate	Age at last egg	.282	.094	.195	.543	.315	.289
	Age at first egg; age at last egg	Winter rate	.331	.125	.243	.537	.132	.130
	Age at last egg; spring rate	Age at first egg	.405	.198	.197	.510	.249	.258
	Age at first egg; spring rate	Age at last egg	.362	.159	.162	.455	.226	.235
	Age at first egg; age at last egg	Spring rate	.633	.424	.458	.469	.298	.312
	Age at last egg; summer rate	Age at first egg	.354	.144	.240	.428	.190	.240
	Age at first egg, summer rate	Age at last egg	.230	.086	.162	.513	.282	.350
	Age at first egg; age at last egg	Summer rate	.412	.202	.319	.365	.129	.174
Biological laying year production	Age at last egg; winter rate	Age at first egg	.578	.341	.305	.775	.465	.185
	Age at first egg; winter rate	Age at last egg	.625	.417	.397	.844	.707	.454
	Age at first egg; age at last egg	Winter rate	.456	.193	.170	.701	.260	.089
	Age at last egg; spring rate	Age at first egg	.673	.444	.272	.530	.288	.275
	Age at first egg; spring rate	Age at last egg	.706	.523	.358	.662	.407	.416
	Age at first egg; age at last egg	Spring rate	.657	.466	.296	.181	.158	.156
	Age at last egg; summer rate	Age at first egg	.582	.336	.312	.585	.352	.260
	Age at first egg; summer rate	Age at last egg	.569	.375	.358	.751	.558	.523
	Age at first egg; age at last egg	Summer rate	.431	.224	.203	.378	.132	.102

The coefficient of part correlation differs from the first measure in that the adjustment for variation is made only in the dependent factor. The coefficient of partial correlation may be said to measure the relationship between the net variation of the independent factor from which the variation due to other independent factors is removed and the net variation of the dependent factor, similarly adjusted. The coefficient of part correlation, on the other hand, measures the relationship between the latter and the gross or unadjusted variation of the independent factor considered. It is calculated from formula 51 of Ezekiel, and the squared values of it appear in table 6 in the columns headed "Coefficient of part determination."

The third method used involves the partial beta coefficients, which represent the coefficients of the regression equations placed on a comparable basis with each other by expressing them not in the units in which they were originally stated but in terms of their respective standard deviations. To avoid negative values the squares of these coefficients rather than the coefficients themselves as determined by Ezekiel's formula 52 are presented in table 6.

The values obtained by these three methods do not always give the same order of magnitude for the different variables, but general trends can be deduced from examination of the order in which they appear for the different regression equations. Thus perusal of table 6 brings out the fact that when the biological laying year production is considered, age at last egg is the single factor of greatest importance. Except in one instance, that of the 1933 series partial correlation coefficient when summer rate is used as the measure of intensity, higher values are obtained for age at last egg than for age at first egg or any of the three rate measures used. Age at first egg undoubtedly seems to be the factor next in importance, the rate measures showing the lowest values throughout with a minor exception in the case of the 1933 series, where spring rate gives a somewhat higher value than does age at first egg.

When the 365-day production is considered the picture is not as clear-cut. Age at last egg still may be considered as the most important factor, although in a number of cases it appears to be somewhat less important than rate. Age at first egg gives uniformly the lowest values for the 1933 series, but shows greater influence on the egg record in the 1934 series.

So far as the relative value of the three measures of rate is concerned, it is hard to pass judgment on the basis of table 6 alone. However, comparison of the data from this table with those of table 4 definitely establishes the point that summer rate is the least adequate of the three measures. Spring rate seems somewhat superior to winter rate, although in a number of cases, particularly when 365-day production in the 1934 series is considered, winter rate accounts for a greater percentage of variance than does spring rate when age at first egg and age at last egg have been already accounted for.

In general it may be considered as established that in this nonpausing population the time factors are of greater importance than the rate factor. Of the factors considered age at last egg, measuring persistency, is the greatest single factor affecting the expression of the inherent ability of a bird to lay eggs. The significance of this is enhanced by the fact that Asmundson (*1*) using similar methods of analysis found that this observation also holds true for turkeys, when date of last egg is used as a measure of persistency.

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CONCLUSIONS AND SUMMARY

A biometric analysis of the egg production records of two selected nonpausing population of Single-Comb White Leghorn pullets reveals that:

(1) 55.3 to 91.4 percent of the variance in annual egg record as measured by 365-day or by biological laying-year production can be accounted for by the variation in age at first egg, age at last egg, and winter or spring or summer and fall rate.

(2) The variance of these factors is to a considerable degree genetic in nature.

(3) Of the factors considered age at last egg is the most important single factor affecting egg production.

(4) Net summer and fall rate of production is not an adequate measure of intensity, the use of spring or winter rate giving more accurate estimates of the annual production.

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